"ANTI-BACTERIAL EFFICACY OF SPILANTHES ACMELLA ON SALIVARY MUTANS STREPTOCOCCI IN 15-17 YEARS OLD SCHOOL STUDENTS IN MADURAI DISTRICT – A RANDOMIZED CONTROLLED TRIAL (RCT)"

Dissertation submitted

in partial fulfillment of the requirements

for the degree of

MASTER OF DENTAL SURGERY

BRANCH – VII

PUBLIC HEALTH DENTISTRY

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI – 600 032

2015 – 2018

DECLARATION BY THE CANDIDATE

I hereby declare that this research entitled **''Anti-bacterial efficacy of Spilanthes acmella on salivary mutans streptococci in 15-17 years old school students in Madurai district- A Randomized controlled trial (RCT) "** is a bonafide and genuine research work done by me under the guidance of **Dr. MUTHU KARUPPAIAH.R, M.D.S.,** Reader, Department of Public Health Dentistry, Best Dental Science College, Madurai.

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The writing of this dissertation has been one of the most significant academic challenges I have ever had to face. Without the support, patience and guidance of the following people, this study would not have been completed on time. It is to them that I owe my deepest gratitude.

First and foremost, my praises and thanks are hailed to the God, the Almighty, for His showers of blessings throughout my research work to complete the research successfully.

I would like to thank our honorable Chairman **Prof. Dr.K.R.Arumugam** and our vice-Chairman **Prof.Dr.A.Babu Thandapani** for their valuable guidance and support throughout this research "The fruitful work of a teacher will be etched in a student's life; beyond the sands of time". I offer my sincere thanks to the Principal **Dr. Vijayalakshmi,K. M.D.S.** and Vice Principal **Dr. Prem Kumar.K.S, M.D.S.** Best Dental Science College, Madurai for their assistance as ethical committee heads and for their consistent guidance and unwavering support throughout this project.

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Lastly, I would like to thank my family for all their love and encouragement. For my parents **Mr.Gunasekaran.K** and **Mrs.Inbavalli.S** who raised me with love and supported me in all my pursuits. I like to thank my sisters **Dr.Nithya BhimRao M.B.BS, Dr.Vidhya.G, (IInd year PG,PHD)Dr. Sudha.G (IInd year PG,OMFP)**for their patience, support and blessings. And most of all for my loving, supportive, encouraging **friends** whose faithful moral support during my career is so appreciated. I wish to thank my Nephew **Mst.Jai Adhithya.B, Mst.Pavan.B.** Thank you.

Finally, my thanks go to all the people who have supported me to complete the research work directly or indirectly.

"This impossible task is made possible by God, „a great power of faith" who showered

his all blessings throughout my carrier and academic life."

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DECLARATION

TITLE OF THE DISSERTATION	**"Anti-bacterial efficacy of Spilanthes acmella on salivary Mutans Streptococci in 15-17 years old school students in Madurai district – A Randomized Controlled Trial (RCT)"**
PLACE OF STUDY	**BEST DENTAL SCIENCE COLLEGE, MADURAI – 625104.**
DURATION OF THE COURSE	**3 YEARS**
NAME OF THE GUIDE	**Dr. MUTHU KARUPPAIAH. R,M.D.S.,**
HEAD OF THE DEPARTMENT	**Dr. BHARATH KUMAR GARLA M.D.S.,**

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance/any promotion without obtaining prior permission of the Principal, Best Dental Science College, Madurai – 625104. In addition, I declare that no part of this work will be published either in print or in electronic media without the guide who has been actively involved in dissertation. The author has the right to reserve for publish of work solely with the prior permission of the Principal, Best Dental Science College, Madurai – 625104.

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Shobana. G.

Date: 29.11.2017

Place: MADURAI

Signature of the Candidate

(DR. SHOBANA.G)

TRIPARTITE AGREEMENT

This agreement herein after the "Agreement" is entered into on this day Dec 2017, between the Best Dental Science College represented by its **Principal** having address at Best Dental Science College, Madurai – 625104, (hereafter referred to as, 'the College')

And

Dr. MUTHU KARUPPAIAH.R, aged 38 years working as **Reader** in Department of Public Health Dentistry at the College, having residence address at No.78, Minikiyur, Kovilpatti post, Manapparai Taluk, Trichy District- 621 305 (herein after referred to as the 'Principal Investigator')

And

Dr. SHOBANA.G aged 27 years currently studying as **Post Graduate student** in Department of Public Health Dentistry, Best Dental College, Madurai- 625104 (herein after referred to as the 'PG/Research student and co-investigator')

Whereas the PG/Research student as part of her curriculum undertakes to research on **''Anti-bacterial efficacy of Spilanthes acmella on salivary Mutans Streptococci in 15-17 years old school students in Madurai District – A Randomized Controlled Trial (RCT)''** for which purpose PG/Principal Investigator shall act as Principal Investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG/Research student as to the extent possible as a Co-investigator.

Whereas the parties, by this agreement have mutually agreed to the various issues including in particular the copyright and confidentiality issues that arise in this regard.

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1. The parties agree that all the Research material and ownership therein shall become the vested right of the college, including in particular all the copyright in the literature including the study, research and all other related papers.
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7. The Principal Investigator shall suitably guide the Student Research right from selection of the Research Topic and Area till its completion. However the selection and conduct of research, topic and area of research by the student researcher under guidance from the Principal Investigator shall be subject to the prior approval, recommendations and comments of the Ethical Committee of the College constituted for this purpose.

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9. If any dispute arises as to the matters related or connected to this agreement herein, it shall be referred to arbitration in accordance with the provisions of the Arbitration and Conciliation Act 1996.

In witness whereof the parties herein above mentioned have on this day, month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

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PG Student: Shobana. G.

Witnesses:

1.

2.

List of abbreviations

S.NO.	ABBREVIATION	MEANING
1.	a.m	Anti- meridian
2.	ANOVA	Analysis of variance
3.	CFU	Colony forming unit
4.	DMFS	Decayed, missing, filled surfaces
5.	DMFT	Decayed, missing, filled teeth
6.	µl	Microliter
7.	ml	Millilitre
8.	mg/ml	milligram/millilitre
9.	MIC	Minimum inhibitory concentration
10.	MIB	Minimum inhibitory bacteria
11.	PLI	Plaque index
12.	SD	Standard deviation
13.	SPSS	Statistical package for social science
14.	WHO	World Health Organisation

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TAXONOMY

SPILANTHES ACMELLA - ANTI TOOTHACHE PLANT

Kingdom	: Plantae
Phylum	: Tracheophyta
Class	: Magnoliopsida
Order	: Asterales
Family	: Asteraceae
Subfamily	: Mimosoideae
Genus	: Acmella
Species	: A. oleracea

INTRODUCTION

INTRODUCTION

Most common infectious disease of oral cavity encountered by many individuals are dental caries and periodontal diseases at different stages of their life time. Dental caries is considered as the most wide spread infectious disease in the world. WHO's report on the Global Problem of Oral Diseases, notes that oral diseases such as dental caries (tooth decay), periodontitis (gum disease) and oral and pharyngeal cancers are global health problem in both the industrialized and the developing countries, especially among poorer communities. Dental caries is a major oral affliction in developing countries, affecting 60-90% of the school children and the vast majority of adults.[1] This alarming trends is of great concern from a public health stand point. Dental caries is a multifactorial infectious disease in which the active agent or agents are members of the indigenous oral flora. Oral microbes can adhere to surfaces throughout the oral cavity. The oral cavity contains a wide variety of oral bacteria, but only a few species of bacteria are believed to cause dental caries (Mutans streptococcus and Lactobacilli). The elimination of cariogenic bacteria from the oral cavity using antibacterial agents is one of the primary strategies for prevention and progression of dental caries.[2] Reducing streptococci load in the oral cavity is recognised as practical step forward to lower the incidence of dental caries.

Various mouthrinses are available in the market, amongst which chlorhexidine is the most popular and accepted widely as a gold standard.[3] It is recognized as the primary agent for chemical plaque, recognized by the pharmaceutical industry as the control against which the efficacy of alternative antiplaque agents should be measured. But it cannot be used on a long term basis because of various side effects like brown staining, taste perturbation, oral mucosal lesions, parotid swellings, enhanced supra gingival plaque formation and sometimes unacceptable taste.[4]

An effective alternative to chlorhexidine with all the good qualities and its unpleasant effects is highly desirable and has been long awaited. Herbal mouthwashes may act as good and cost effective oral hygiene product. The research for natural products to cure disease represents an area of great interest in which plants have been the most important source. Most of the herbal plants exhibit anti-microbial activity due to the production of sesquiterpene as principal secondary metabolites. Extracts from siwak, green and black tea, garlic etc., have proved to be effective against S.mutans. Herbs are being widely explored to discover alternatives to synthetic antibacterial agents.[2] This research for the first time introduces and explores the herbal plant "Spilanthes acmella" (ANTI TOOTHACHE PLANT). In Tamil, it named as "Palvazhi poondu chedi", brazil named as "jambu"

Spilanthes acmella is a small herb belonging to family Compositae (para-cress or toothache plant) is unique & versatile plant with a renowned reputation as toothache plant. The name toothache plant comes from the numbing property it produces when the leaves and flowers are chewed (Jansen 1985). The plant has long been used in India for the treatment of gum & dental problems.[5] The antibacterial usuage greatly affect the number of resistant organisms. The resistance problem demands that a renewed effort be made to seek anti-bacterial agents effective against pathogenic bacteria resistant to current antibiotics. One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals. Many of the herbs and spices used by humans to have useful medicinal compounds including those having antibacterial activity. Plant derived drugs remain an important resource especially in developing countries to combat serious diseases.[6]

Spilanthes is a genus comprising of over 60 species that are widely distributed in tropical and subtropical regions of the world, such as Africa, America, Borneo, India, SriLanka and Asia. Spilanthes acmella is native to Brazil and is cultivated throughout the year as ornamental or medicinal plant.[7] The local and tribal people of flowers of Spilanthes acmella and are chewed to relieve toothache. The increasing demand on herbal medicines and their acceptance in international market, because of high potential pharmacological agent and high therapeutic value have been proving to be real blessing to the people. However, efforts are needed to explore, standardise, and validate ayurvedic medicines for their potency, safety and efficacy in order to bring them to market as main line therapeutics.[8] The whole plants (e.g. flowers, leaves, roots, stems and aerial parts) of Spilanthes acmella have been used in health care and food sectors. Particularly, Spilanthes acmella is a well-known anti-toothache plant and has been used as traditional medicine.

However it was observed in literature only methanol extracts of leaves and flowers of Spilanthes acmella showed zone of inhibition and no zone of inhibition were seen for chloroform and hexane extracts in in vitro studies.[5]

The present research introduces the first in vivo study to test, "Antibacterial efficacy of Methanolic extract of Spilanthes acmella may be due to Methanol or Spilanthes acmella or synergistic action of both."

These opens a scope for the clinical trial to test the antibacterial efficacy of Spilanthes acmella on salivary Mutans Streptococci.

AIMS AND OBJECTIVES

AIM AND OBJECTIVES

AIM:

To assess the antibacterial efficacy of Spilanthes acmella on salivary Mutans Streptococci.

OBJECTIVES

1) To assess an antibacterial efficacy of plant extract on Mutans Streptococci level.
2) To assess an antibacterial efficacy of methanol on Mutans Streptococci level.
3) To assess an antibacterial efficacy of chlorhexidine on Mutans Streptococci level.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

In 2010, Fouad Hussein Al-Bayaty et al., conducted clinical study aimed to Salvadora persica (Miswak) was extracted with 60% ethanol and was examined for its toxic effect, assessed its antibacterial activity and evaluated clinically for its effect on dental plaque formation. A 4 day plaque regrowth, double - blind, crossover design was used in which 10 dental students volunteers were rendered plaque free (0.3), ceased tooth cleaning, then, asked to rinse twice daily for 1.5 min each time with 10 ml of chlorhexidine 0.2% mouth rinse and three times daily for 1.5 min each time with 10 ml of S. persica 10% solution and placebo mouth rinse. On day five, plaque was scored by the plaque index system (PLI). A wash out period of 2 days was allowed in which the volunteers returned to self- performed plaque control, then a new test period was initiated. Statistical analysis showed that the mean PLI score were 1.48 for S. persica mouth rinse, 0.48 for chlorhexidine and 2.07 for placebo mouth rinse. Acute toxicity test revealed no mortality among the experimental animals which is an indication that S. persica crude extract solution is well tolerated, disk diffusion test showed a marked antibacterial effect in vitro and this effect is concentration dependent, had an effect in in vivo, but this effect cannot be considered absolute.

In 2012, Veda Prachayasittikul et al., in their review have described that Spilanthes acmella, a well known anti toothache plant with high medicinal usages, has been recognized as an important medicinal plant and has an increasingly high demand worldwide. Traditional uses, applications and dental application of S. acmella. In brief, it could be demonstrated that S. acmella is a medicinal plant enriched with compounds having high therapeutic value that can be further developed for applications in medicines, health care, cosmetics, supplements and health food.

In 2012, Thompson et al., conducted an in vitro study that to discover the antibacterial effect of Acmella oleraceae against dental caries bacteria and aware the populace about the importance of using phytomedicines.by this study, the antibacterial effects of Acmella oleraceae was checked against the dental caries bacteria using well diffusion method. Phytochemical screening of Acmella oleracea checked with chloroform, ethanol, methanol and water and acetone extracts. Acetone extract of Acmella oleracea showed high activity against Neisseria catarrhalis and Streptococcus mutans. Chloroform extract revealed high effect over Streptococcus viridans . Methanol extract showed high activity against Bacillus megaterium and Pseudomonas aeruginosa. But the water extract did not showed any inhibitory effect over the test organisms. And concluded that Acmella oleraceae is capable and best remedy to inhibit the growth of dental caries bacteria with its copious source of secondary metabolites.

In 2013, Pallavi P. Borate et al., conducted an in vitro study to evaluate the activity of flowerheads and leaf extracts of Spilanthes acmella against potential pathogens viz Bacillus megaterium, B. subtilis, B. cereus, Escherichia coli, Klebsiella pneumonaie, Micrococcus luteus, Nocardia sp. and Pseudomonas aeruginosa. The plant parts were dissected in to pieces, dried and then mixed with three solvents (methanol, hexane, chloroform) with different concentrations 1%, 3%, 5% and 10%. It was observed that only methanol extracts of leaves and flowerheads of Spilanthes acmella showed zone of inhibition and no zone of inhibition were seen for chloroform and hexane extracts. From this results it can be concluded that methanol extracts of flowerheads and leaves of Spilanthes acmella have good antibacterial activity against tested organisms.

In 2013, Suchita Dubey et al., in their review have described that Spilanthes acmella is an important medicinal plant, found in tropical and subtropical countries mainly India and South America. Popularly, it is known as toothache plant which reduces the pain associated with toothaches and can induce saliva secretion. Various extracts and active metabolites from various parts of this plant possess useful pharmacological activities. Literature survey proposed that it has multiple pharmacological actions, which include antifungal, antipyretic, local anaesthetic, bioinsecticide, anticonvulsant, antioxidant, aphrodisiac, analgesic, pancreatic lipase inhibitor, antimicrobial, antinociception, diuretic, vasorelaxant, anti-human immunodeficiency virus, toothache relieve and anti-inflammatory effects. This review is elaborately describing the traditional uses, phytochemistry, pharmacology, and toxicology of this plant.

In 2013, Bipul Biswas et al., conducted an in vitro study, aimed to determine the antimicrobial potential of guava (Psidium guajava) leaf extracts against two gram-negative bacteria (Escherichia coli and Salmonella enteritidis) and two gram-positive bacteria (Staphylococcus aureus and Bacillus cereus) which are some of foodborne and spoilage bacteria. The guava leaves were extracted in four different solvents of increasing polarities (hexane, methanol, ethanol, and water). The efficacy of these extracts was tested against those bacteria through a well-diffusion method employing 50μL leaf-extract solution per well. According to the findings of the antibacterial assay, the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts. The methanol extract had an antibacterial activity with mean zones of inhibition of 8.27 and 12.3 mm, and the ethanol extract had a mean zone of inhibition of 6.11 and 11.0 mm against B. cereus and S. aureus respectively. On the basis of the present finding, guava leaf-extract might be a good candidate in the search for a natural antimicrobial agent. This study provides scientific

understanding to further determine the antimicrobial values and investigate other pharmacological properties.

In 2014, Jyotsna Srinath et al., in this review described that Spilanthes acmella has many dental uses. It can be used to treat toothache, in the treatment of periodontitis and aphthous ulcers. The characteristic feature of this plant is that its flowerhead has a numbing effect which helps in treating toothache. Noor Jahen et al demonstrated that the ethanolic extract of S.acmella showed antimicrobial activity against Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi, Shigella dysenteriae the extract also showed activity against resistant bacteria. Agar dilution assay of S.acmella also showed activity against 27 strains of bacteria both gram positive and gram negative. And concluded the study that Spilanthes acmella is an ornamental plant with high therapeutic benefits like diuretic, antifungal, treatment of rheumatoid arthritis, antimalarial etc. It also has many dental uses. It can be used to treat toothache, in the treatment of periodontitis and aphthous ulcers. The characteristic feature of this plant is that its flower head has a numbing effect which helps in treating toothache.

In 2015, H.A.Thakur et al., conducted an in vitro study to find out the Preliminary phytochemical analysis and antimicrobial activity of the different extracts of entire plant including flower heads of Spilanthes acmella. The different extracts such as Hexane, Methanol, Ethanol, petroleum ether and aqueous extracts exhibits comparable antimicrobial activity with the control. Out of this five extracts, hexane, showed high in vitro antibacterial activity against S. aureus, hexane extract showed strong antibacterial activity against E. coli, S aureus. C. albicans fungal pathogen strongly inhibited by ethanol and hexane extract and none of antimicrobial activity found against bacteria

K. pneumonia, Distilled water extract and pure solvent used as control not showed any activity against micro organism.

In 2015, L.G.Vijayaalakshmi et al., conducted an in vitro study, aimed to estimate the antibacterial effect of herbal mouth wash in comparison with conventional mouth wash in reducing streptococcus mutans count. Isolated colonies of Streptococcus mutans was prepared for an antimicrobial mouth rinse test. The tube dilution method was used for determining the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). The use of herbs in dentistry should be based on evidence of effectiveness and safety. The anti-bacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration. The present results therefore offer a scientific basis for traditional use of herbal mouth wash

In 2015, Meena Priya et al., conducted an in vivo study aimed to compare the efficacy of green tea and chlorhexidine mouth rinses in the control of plaque and gingival inflammation. This study concluded that both the mouthwashes were equally effective in reducing plaque and gingival inflammation, considering the fact that the chemical formulations of commercially available mouth rinses are chemically based, expensive, and have considerable side effects, which restricts their use. India has a rich source of herbal plant products with medicinal value. Green tea can be used as adjuvant to oral hygiene maintenance with a goal on the prevention as well as the prevalence of periodontal diseases due to its antibacterial and antioxidant properties.

In 2015, Mahesh et al., conducted an in vivo study aimed to compare the effect of 0.2% Chlorhexidine mouthwash and 0.6% Cranberry mouthwash on undergraduate dental students.Preparation of Cranberry mouthwash was done. The entire sample size of 50 subjects were randomly divided into Group A (25 subjects) who were given Chlorhexidine

mouthwash® (Welldent, Purple Remedies, Ahmedabad, Gujarat, India) (designated as A) and Group B (25 subjects) who were given Cranberry mouthwash (designated as B) employing lottery method. The study concluded that Cranberry can prove to be effective or better alternatives to Chlorhexidine in improving the oral health with added systemic benefits and minimal side effects.

In 2016, Dilshad Umar et al., conducted an in vivo study aimed to estimate the influence of pomegranate extract mouthrinse on salivary pH and the S. mutans count and To compare the antibacterial and anticaries effect of a pomegranate extract mouthrinse with chlorhexidine. This study concluded that This study implies that, PPE mouthrinse possesses remarkable antimicrobial activity against S. mutans present in the oral cavity as tested in vivo, and may be used as an adjunct to prevent dental caries and maintain good oral hygiene.

In 2016, Sajjan shetty et al., conducted an in vitro study aimed to conceptualized as the initial step to comprehensively report the antimicrobial potential of Tulsi by assessing the inhibition zones with agar well diffusion method against three pathogens viz., *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*, which are commonly associated with periodontitis. The findings were then compared with those obtained with doxycycline, a drug used commonly as an adjunct to nonsurgical therapy for treatment of periodontitis, as a standard.the study concluded that Tulsi, as an adjunct, "if" found effective and safe on further research would be considered as a potential "adjunct" along with the standard care in the management of periodontitis to overcome the side effects of synthetic drugs, especially in this era of ever advancing clinical dentistry.

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DESIGN:

The present research is a randomized, concurrent parallel arm, controlled trial designed to test the ‘‘Anti-bacterial efficacy of Spilanthes acmella on salivary mutans Streptococci.

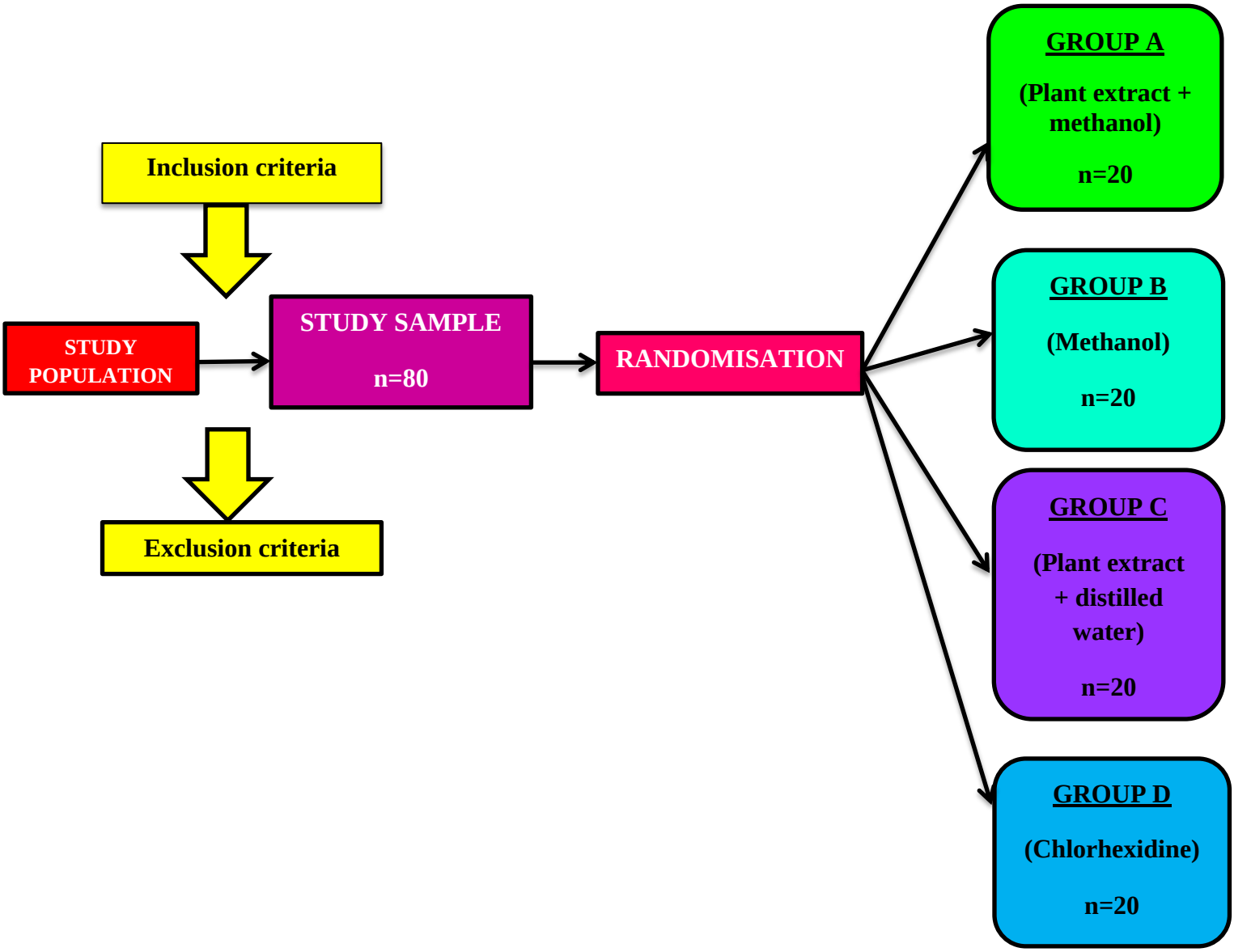


Figure 2: Study design - parallel arm, Randomised controlled trial with four groups

STUDY AREA

The Madurai district is the ninth largest in population of the 32 districts of the state of Tamil Nadu in southeastern India. Madurai is called with various nicknames like *Athens of the East, Koil Nagar* (Temple city) etc. The history of Madurai is intertwined with the history of the Tamil Language and the third Tamil Sangam, a chief assembly of Tamil scholars, is believed to have been conducted in the city.

ETHICAL CLEARANCE

Ethical clearance was obtained from Institutional Review Board. Permission for the research was granted by school authorities.

PERMISSION BY THE SCHOOL AUTHORITIES

The head of the institutions were approached by Principal investigator. The aim, objective and methodology of the research was explained to the school authority. Before start of study, mouthwashes were distributed to the authority, used for one week and permission was sought to conduct the research among the students. In the class rooms, the methodology of the research was explained to the students. Only those who consented to participate in the study were included.

INFORMED CONSENT

Written consent in local language from the parents and assent from the subjects was obtained before the start of the study.

STUDY POPULATION

The study population include school going children of aged 15 – 17 years old, attending higher secondary school educational institutions in Madurai.

ELIGIBILITY CRITERIA

INCLUSION CRITERIA

1. The subjects with atleast one active caries lesion.

2. The subjects with gingival and or/ periodontal disease

3. The subjects who brush only once daily.

EXCLUSION CRITERIA

1. Patients who received antibiotic therapy within past 3 weeks.

2. The subjects who had preventive dental procedures at last 48 hours.

3. The subjects those who had use mouth wash for past one month.

4. The subjects with limited manual dexterity and systemic illness.

5. Patients giving known history of allergy to any of the constituents of mouth rinses.

SOURCE OF DATA

Primary data which includes clinical examination and stimulated saliva collection for microbiological analyses.

PREPARATION OF MEDIA

Mitis Salivarius Agar Medium

Mitis Salivarius Agar is recommended for the isolation of streptococci.

Composition of Media

Ingredients	gm/liter
Casein enzymic hydrolysate	15.000
Peptic digest of animal tissue	5.000
Dextrose	1.000
Sucrose	50.000
Dipotassium phosphate	4.000
Trypan blue	0.075
Crystal violet	0.0008
Agar	15.000
Final pH (at 25°C)	7.0±0.2

Preparation of medium:

Suspend 90.07 grams of Mitis Salivarius Agar in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50-55°C and add 1 ml of sterile 1% Potassium Tellurite Solution (FD052). Do not reheat the medium after the addition of tellurite solution. Mix well and pour into sterile Petri plates.

Preparation of 48 hours culture

Toothbrushes were immersed and incubated in tubes containing 5ml phosphate-buffered saline solution (PBS, pH 7.2) at 37°C under anaerobic conditions for 48 hours. After

inhibition 25µl aliquots of the specimens were seeded onto nutrient agar. The specimens were incubated as mentioned for 48 hours and the grown colonies were counted. The number of colonies was calculated according to dilution ratio and defined as the number of colony forming units (CFU) per milliliter.

This procedure was standardised to carry out pilot and min research.

Formula

$$\text{CFU/ml (cfu/ml)} = \frac{\text{Number of colonies} \times \text{dilution factor } (10^3 = 1000)}{\text{Volume of culture plate (25µl convert into ml = 0.025ml (25/1000)}}$$

$$\text{CFU/ml (cfu/ml)} = \frac{\text{Number of colonies} \times 1000}{0.025\text{ml}}$$

ASSESSMENT OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The different concentrations of sample dilutions (1,3,5,10 and 100 %) were prepared. They were kept under refrigerated condition unless they were used for the experiment. Standard solution as chlorhexidine used to compare the test solution.

Measurement of zone of inhibition

The antimicrobial potential of test compounds were determined on the basis of mean diameter of zone of inhibition around the disc in millimetre scale.

Table 1: Assessment of MIC in different concentrations.

Concentrations	**50 µl**	**100 µl**	**150 µl**	**200 µl**	**Control (Methanol 30 µl)**
1 %	Nil	Nil	Nil	Nil	Nil
3 %	Nil	Nil	Nil	0.50±0.03	Nil
5 %	Nil	Nil	Nil	2.20±0.08	Nil
10 %	Nil	Nil	2.70±0.04	3.50±0.10	Nil
20 %	1.80±0.04	3.10±0.07	4.40±0.11	5.30±0.16	Nil
Chlorhexidine (Std.)	2.40±0.16	3.20±0.22	4.60±0.32	5.90±0.41	-------

Values are expressed as mean ± standard deviation.

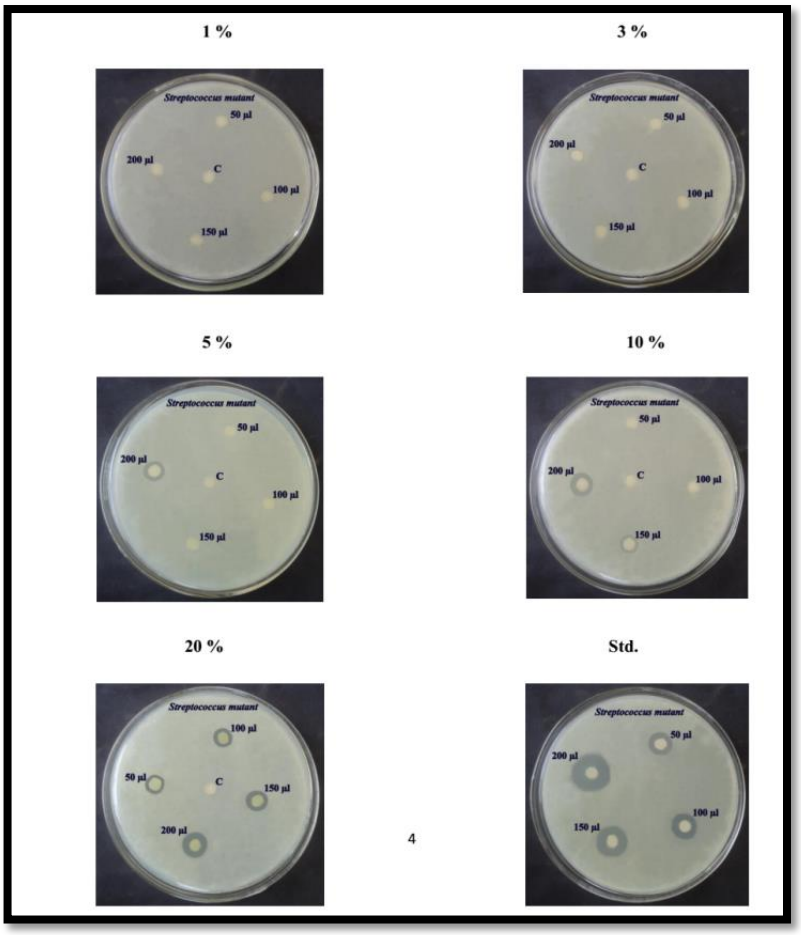


Fig.3: Assessment of MIC in different concentrations

Based on this in vitro study results, the final dose was 20% in dose dependent manner, this dose shows equally efficacious with chlorhexidine mouthwash.

EXAMINER CALIBRATION

Training exercises were first carried out in the Department of Public Health Dentistry, Ultra‟s Best Dental Science College and Hospital on the out-patients under the guidance of a trained person. About twenty subjects were examined to assess the consistency of intra-examiner reproducibility. The agreement for most assessments was expected to be 90%.

PILOT STUDY

Pilot study was conducted in Milton matriculation higher secondary school in the month of July 2017. 20 subjects, 5 in each group were selected based on inclusion and exclusion criteria. Pilot study aimed to check the feasibility, practical issues like the acceptability, palatability and any immediate side effects of the mouth rinses by the subjects, saliva collection, the time taken to transport the samples to the lab, microbiological analyses of the samples, were all tested during this pilot study.

Table 2: Percentage reduction of Mutans Streptococci in four groups.

		Group			
		Group-A	Group-B	Group-C	Group-D
Percentage Reduction	n	5	5	5	5
	Mean	63.72	11.51	-17.28	64.60
	Std Dev	7.32	48.99	37.91	9.08
	Minimum	54.58	-63.86	-66.28	53.69
	1st Quartile	58.47	-10.89	-35.37	58.64
	Median	64.10	31.63	-22.89	62.99
	3rd Quartile	69.07	50.00	5.88	72.80
	Maximum	**72.35**	50.64	32.26	**74.88**

Pilot result shows maximum reduction in Group D, Group A succeeded by Group B and Group C in minimum reduction.

Therefore, 50% reduction shows in Group A, Group B, and Group C.

SAMPLE SIZE DETERMINATION

The sample size for the present study was determined scientifically. Based on pilot results sample size was estimated. The required sample size in each group was calculated using

F tests - ANOVA: Fixed effects, omnibus, one-way

Analysis: A priori: Compute required sample size

Input:	Effect size f	=	0.7126760
	α err prob	=	0.05
	Power (1-β err prob)	=	0.90
	Number of groups	=	4
Output:	Noncentrality parameter λ	=	18.2846549
	Critical F	=	2.9011196
	Numerator df	=	3
	Denominator df	=	32
	Total sample size	=	36
	Actual power	=	0.9364057

The required sample size with 90% power is 9 per group and in total 36.

Making an allowance of attrition, the required sample size for each group is calculated to be 20.

Total sample size for the two groups = 4 x 20 = 80

The required sample size is 80 cases for a significance level of 0.5 % (confidence level of 95%) and power of 90%.

SAMPLING METHODOLOGY

After obtaining permission from the Principal of the school, a screening camp was conducted in the school premises to screen all the students. A list was prepared after the initial screening examination of all students in the school. The principal investigator maintained this "master list" of all those students in the college who were eligible to participate in the study based on the inclusion and exclusion criteria. Using "simple random sampling" students were randomly picked from this list to participate in the study based on their availability.

RANDOMIZATION

Blocked randomization was carried out throughout this research to reduce howthorne effect. Mouthwash was distributed to the respective groups. The enrollment was stratified by gender to ensure that equal number of males and females were allocated to each group (n=10 males and 10 females in each group).

COLLECTION OF DATA

MATERIALS USED IN THE STUDY

1. Diagnostic instruments: mouth mirrors , tweezers and No:23 Explorers
2. Sterile Gloves and Mouth masks.
3. Sterilized rubber bands
4. Sterile containers for collection of salivary samples.
5. Cold storage box with ice packs.
6. Stopwatch.
7. Data recording forms.
8. Plant extract and methanol mouthwash
9. Methanol mouthwash

10. Plant extract and distilled water mouth
11. 0.2% Chlorhexidine gluconate
12. Culture media for mutans streptococci. M259-Mitis Salivarius Agar with 1% potassium tellurite (HiMedia, Batch number: 228506) CMS208 Bacitracin (HiMedia, Batch number: 249178).

INTERVENTIONS

The plant material

Fresh plant parts i.e, leaves of Spilanthes acmella are collected from the garden of Agriculture Department in Thanjavur district. Plant parts were washed thoroughly in running tap water, rinsed with distilled water, dissected into pieces, air dried at room temperature, powdered and stored as stock powder in air tight amber coloured bottles.

PREPARATION OF PLANT EXTRACT + METHANAOL AS MOUTHWASH (GROUP A)

Solvent extraction of active components

20 gram stock powdered leaves of Spilanthes acmella was mixed with 80ml of organic solvent ie.,methanol, separately in a conical flasks (soxhlet apparatus) and after plugging, the material was kept shaking at 190-220 rpm on Remi metabolic shaker for 72 hours. After 72 hrs, the mixtures were filtered through muslin cloth filter and centrifuged at 5000 rpm for 15mins. The supernatants were collected and the solvents were evaporated to make the final volume as 20 ml range and add the ingredients of polysorbate 80, sodium saccharine, menthol and propylene glycol.

PREPARATION OF METHANOL AS MOUTHWASH (GROUP B)

20 ml of methanol (organic solvent) kept separately in a conical flasks and mixed with 80 ml of distilled water, the material was kept shaking at 190-220 rpm on Remi metabolic shaker for 72 hrs. After 72 hrs, the solvents were evaporated to make the final volume as 5% of methanol to be used. Add the ingredients of polysorbate 80, sodium saccharine, menthol and propylene glycol.

PREPARATION OF PLANT EXTRACT + DISTILLED WATER AS MOUTHWASH (GROUP C)

20 gram stock powdered leaves of Spilanthes acmella was mixed with 80ml of distilled water separately in a conical flasks and after plugging, the material was kept shaking at 190-220 rpm on Remi metabolic shaker for 72 hours. After 72 hrs, the mixtures were filtered through muslin cloth filter and centrifuged at 5000 rpm for 15mins, the supernatants were collected and the solvents were evaporated to make the final volume as 20 ml range and add the ingredients of polysorbate 80, sodium saccharine, menthol and propylene glycol.

0.2% CHLORHEXIDINE GLUCONATE MOUTHWASH (GROUP D)

Commercially available 0.2% Chlorhexidine gluconate mouthwash (CLOHEX PLUS, Dr.Reddys Laboratories LTD., Batch No. BCP 7011)) was used as positive control. Chlorhexidine was chosen as positive control as it is hailed as the "gold standard" mouthwash. Chlorhexidine exhibits both bactericidal and bacteriostatic effects depending on the concentration. However, the property of substantivity is a unique characteristic of this chemical plaque control agent. Previous researches have documented the initial suppression of Mutans Streptococci in the saliva after administration of chlorhexidine.

Toxicity

Herbal medicines are believed to be benign and to not cause severe toxicity. Spilanthes acmella plant doesn't show any adverse effect and toxicity throughout the study.

Shelf life

Shelf life is the length of time that a commodity may be stored without becoming unfit for use, consumption, or sale. Spilanthes acmella mouthwash was analysed for one year. For first three months, active ingredients/phytochemicals cannot be altered. this says mouthwash was preserved for three months. In present study, mouthwash was distributed for four weeks.

Dosage

The extract of poly-herbal powders was formulated by using pharmaceutical excipients like soothing and cooling agents (menthol),thickening agent (propylene glycol), sweetening agent(sodium saccharine) into a mouthwash. The formulation was prepared using 20%w/v of poly herbal extract in any single dose of the formulation, to provide optimal antimicrobial activity and palatability.[9]

INFECTION CONTROL

Sufficient numbers of instruments were carried to the site of the trial to avoid any interruption in the examinations. The used ones were washed and drained well before sterilization. Proper use of instruments was ensured to obviate digital manipulation in order to minimize the risk of cross-infection. Disposable gloves and masks were used. The wastes generated during the examination and the trial was disposed appropriately

PREPARATION OF SPECIAL PROFORMA

A special proforma was prepared to collect the required data. The proforma was prepared in English language. The proforma was divided into three different sections.

i. The first section contained provision to record patient's demographic details such as Name, Age/Gender, Address, Phone Number And Socioeconomic Status (according to Kuppuswamy's Classification, 2013 Modification).

ii. The second section contained provision to record oral hygiene practices and other habits.

iii. The third section contained tables to record Decayed, Missing and Filled teeth index and Decayed, Missing, And Filled surfaces Index - (Henry.T. Klein, Carrole. E.Palmer And Knutson. J.W , 1938 – Original Index)

CLINICAL EXAMINATION

A single examiner, the investigator, carried out the clinical examination of all the study subjects involved in the study under artificial light using standardized instruments ADA specification type III examination. Participants were seated comfortably on an ordinary chair and examined. Oral examinations were conducted using a plain mouth mirror and an explorer. Only 20 patients per day were recruited in the research. Dental caries experience was assessed using appropriate armamentarium and Decayed, Missing and Filled teeth index and Decayed, Missing, And Filled surfaces Original Index - (Henry.T.Klein, Carrole.E. Palmer and Knutson. J.W, 1938) were scored.

The criteria for identification of Dental caries according to this index is:

1) The lesion is clinically visible and obvious.

2) The explorer tip can penetrate deep into soft yielding material.

3)There is discoloration or loss of translucency typical of undermined or demineralised enamel.

4)The explorer tip in a pit or fissure catches or resists removal after moderate to firm pressure on insertion and when there is softness at the base of the area.

SALIVA COLLECTION

The purpose and method of saliva collection was explained to the research participants. All saliva samples were collected between 9.30 a.m from the research participants who were clearly instructed to not to consume any food or drinks except water for at least one hour before the sample collection. The participants were asked to sit relaxed in normal chairs and benches. The Stimulated whole saliva was collected by "drooling method." Saliva was stimulated using sterilized rubber bands. A pre sampling time of 1 minute was given for the participant to chew the bands. The participants were instructed to chew the bands and drooled saliva into the sterile labelled containers for the next five minutes. The minutes were timed with a stopwatch. Saliva samples containing visible blood were discarded.

1 ml stimulated saliva added with 4ml of thioglycolate medium in a test tube , carry to the microbial lab analyses. Inoculated and cultured Mutans Streptococci count of all the samples were estimated. Pre and Post rinse saliva samples of one month duration (baseline and final data) were collected in the same manner. The sterile containers were quickly closed

in order to avoid contamination and store in cold storage box and were transported to the Microbiological lab within 2 hours.

DIRECTION FOR USE

The participants were educated and motivated for usage of mouthwash. Further steps to be followed.

Step 1: Pour 10 ml of mouthwash in the cap. Do not dilute with water. The participants were instruct to use a mouthwash after brushing.

Step 2: Empty the cup into your mouth and keep it closed while swishing to prevent the liquid from squirting out.

Step 3: Swish the liquid in your mouth for 30 seconds to 1 minute.

Step 4: Once you're done, spit out the liquid in the sink. Make sure that you don't swallow the liquid as mouthwashes.

EMERGENCY CARE AND REFERRAL

Mouthwash given, complaints was checked on regular intervals. In case of any emergencies that requires immediate attention, the participants were provided with the phone numbers of the Principal investigator and the Guide. Address of the Dental college (Best Dental Science College and Hospital, Madurai) was also provided for referral care.

FEEDBACK FORM

At the end of final data, Principal investigator and Guide were formulated the feedback form, which include five question to understand better about the mouthwash usage.

STATISTICAL ANALYSIS

The information collected regarding all the selected cases were recorded in a Master Chart. Data analysis was done with the help of computer using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, version 22.0 for Windows).

Using this software frequencies and percentages were calculated for qualitative variables. Means and standard deviations were calculated for quantitative variables. ‘t’ value, chi square and 'p' values were also calculated. Student’s ‘t’ test and ANOVA were used to test the significance of difference between quantitative variables and Yate’s and Fisher’s chi square tests for qualitative variables. A 'p' value less than 0.05 denotes significant relationship

Flowchart of Study Procedure

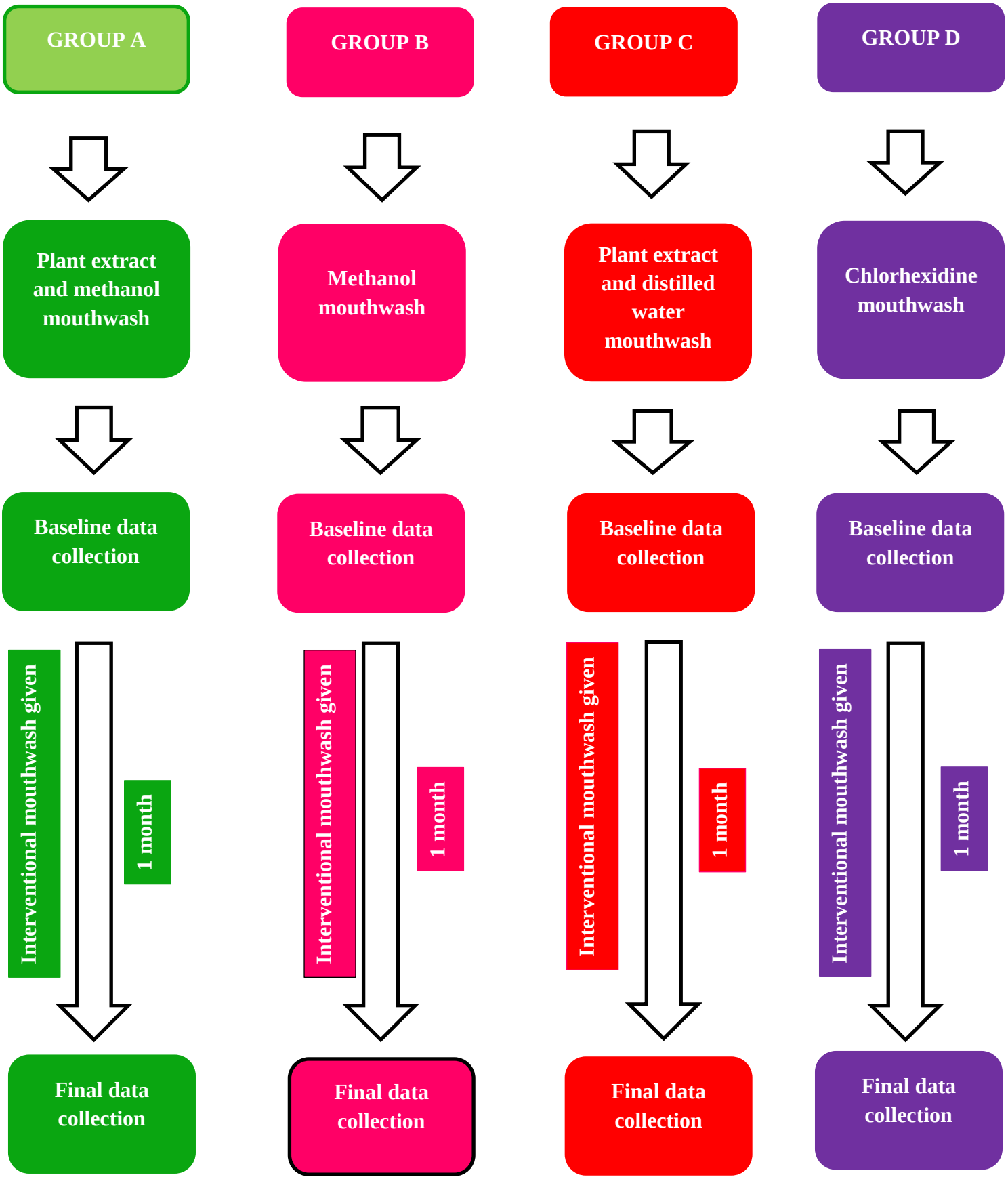


FIG.1: SPILANTHES ACMELLA MOUTHWASH PREPARATION

FIG.2: MILTON MATRICULATION HIGHER SECONDARY SCHOOL

FIG.4: R.V MATRICULATION HIGHER SECONDARY SCHOOL

FIG.5: SEVENTH-DAY ADVENTIST MATRICULATION HIGHER SECONDARY SCHOOL

FIG.6: SAKTHI VIDYALAYA MATRICULATION HIGHER SECONDARY SCHOOL

FIG.7:ARMAMENTARIUM USED

FIG.8: STERILE ELASTIC BANDS FOR SALIVA STIMULATION

FIG.8:STERILE CONTAINER FOR SALIVA COLLECTION

FIG.9: CULTURE MEDIA FOR MUTANS STREPTOCOCCI M259-MITIS SALIVARIUS AGAR WITH 1% POTASSIUM TELLURITE(HIMEDIA,BATCH NUMBER:228506)CMS208 BACITRACIN(HIMEDIA,BATCH NUMBER:249178)

FIG.10:INTERVENTIONAL MOUTHWASH

FIG.11 :PARTICIPANTS WITH INFORMED CONSENT

RESULTS

FIG.12.EXPLAINING ABOUT AIM AND METHODOLOGY TO THE PARTICIPANTS

FIG.13: CLINICAL EXAMINATION

FIG.14: STIMULATION OF SALIVA WITH ELASTIC BAND BY SPITTING METHOD

FIG.15: COLD STORAGE BOX WITH SALIVA SAMPLES AND ICE PACKS

RESULTS

RESULTS

Gender Distribution of the study participants in both the groups (TABLE 3, GRAPH 1)

With respect to the gender of the participants, there was an equal distribution between the groups with n=10(50%) male subjects and n=10(50%) female subjects in each of the four groups. p value was not significant (p=1.0)

Inter group comparison of Mutans Streptococci Count (CFU/ml) values between the four groups before using mouthwash (TABLE 4, GRAPH 2)

p value is not statistical significance seen in all the four groups. There is no statistical significant difference seen when comparing Spilanthes acmella plant extract + methanol mouthwash (Group A) and methanol mouthwash (Group B), Spilanthes acmella plant extract + methanol mouthwash (Group A) and Spilanthes acmella plant extract + distilled water mouthwash (Group C), Spilanthes acmella plant extract + methanol mouthwash (Group A) and chlorhexidine (Group D), methanol mouthwash (Group B) and Spilanthes acmella plant extract + distilled water mouthwash (Group C), methanol mouthwash (Group B) and chlorhexidine (Group D), Spilanthes acmella plant extract + distilled water mouthwash (Group C) and chlorhexidine (Group D).

Inter group comparison of Mutans Streptococci Count (CFU/ml) values between the four groups after using mouthwash (TABLE 5, GRAPH 3)

p value is statistical significant difference seen in all the four groups. There is no statistical significant difference seen when comparing Spilanthes acmella plant extract + methanol mouthwash (Group A) and chlorhexidine (Group D). There is a statistical significance in Spilanthes acmella plant extract + methanol mouthwash (Group A) and methanol mouthwash (Group B), Spilanthes acmella plant extract + methanol mouthwash (Group A) Spilanthes acmella plant extract + distilled water mouthwash (Group C),

methanol mouthwash (Group B) and Spilanthes acmella plant extract + distilled water mouthwash (Group C), methanol mouthwash (Group B) and chlorhexidine(Group D), Spilanthes acmella plant extract + distilled water mouthwash (Group C) and chlorhexidine (Group D).

Inter group comparison of change in Mutans Streptococci Count (CFU/ml) values between the four groups before and after using mouthwash (TABLE 6,GRAPH 4)

p value is statistical significance seen in all the four groups. There is no statistical significance when comparing Spilanthes acmella plant extract + methanol mothwash (Group A) and chlorhexidine (Group D). p value is statistical significance in Spilanthes acmella plant extract + methanol mouthwash (Group A) and methanol mouthwash (Group B), Spilanthes acmella plant extract + methanol mouthwash (Group A) and Spilanthes acmella plant extract + distilled water mouthwash (Group C), methanol mouthwash (Group B) and Spilanthes acmella plant extract + distilled water mouthwash (Group C), methanol mouthwash (Group B) and chlorhexidine(Group D), Spilanthes acmella plant extract + distilled water mouthwash (Group C) and chlorhexidine (Group D).

Changes within Plant extract + Methanol Group (TABLE 7, GRAPH 5)

In Group A (Spilanthes acmella Plant extract + Methanol Group), statistical significant difference seen between before and after using mouthwash. There is maximum (6.34) mean reduction after using mouthwash. 64.8 percentage reduction seen after using this mouthwash.

Changes within Methanol Group (TABLE 8, GRAPH 6)

In Group B (Methanol Group), 4.27 mean reduction after using mouthwash. There is a 50.6 percentage reduction after using mouthwash. Therefore, there is statistical significance seen between before and after using this mouthwash.

Changes within Plant extract + distilled water Group (TABLE 9, GRAPH 7)

In Group C (Spilanthes acmella plant extract + distilled water Group), minimal (1.66) mean reduction after using mouthwash. There is a 19.2 percentage reduction after using mouthwash. Therefore, there is statistical significance seen between when changes within this group before and after using this mouthwash.

Changes within Chlorhexidine Group (TABLE 10,GRAPH 8)

In Group D (Chlorhexidine Group), maximum (6.28) mean reduction seen after using mouthwash. There is a 68.7 percentage reduction seen after using mouthwash. Therefore, there is statistical significance seen between before and after using this mouthwash.

Inter group comparison of percentage of change in Mutans Streptococcus Count (CFU/ml) values between the four groups before and after using mouthwash (TABLE 11,GRAPH 9)

p value is statistically significance seen in all the four groups after using mouthwash. There is no statistical significant when comparing Spilanthes acmella plant extract + methanol mouthwash (Group A) and chlorhexidine (Group D). p value is statistically significant in Spilanthes acmella plant extract + methanol mouthwash (Group A) and methanol mouthwash (Group B), Spilanthes acmella plant extract + methanol mouthwash (Group A) and Spilanthes acmella plant extract + distilled water mouthwash (Group C), methanol mouthwash (Group B) and Spilanthes acmella plant extract + distilled water

mouthwash (Group C), methanol(Group B) and chlorhexidine(Group D), Spilanthes acmella plant extract + distilled water mouthwash (Group C) and chlorhexidine (Group D).

FEEDBACK RESULTS

Did you use mouthwash regularly? (GRAPH 10)

Out of 80 participants, 69 participants used mouthwash regularly. 11 participants in Group c (plant extract and distilled water) not used mouthwash regularly.

How did you feel while using mouthwash? (GRAPH 11)

Group A (Spilanthes acmella plant extract and methanol and Group D (chlorhexidine) participants felt freshens. Most of the participants in Group B(METHANOL) felt no change. In Group C participants felt worsens while using mouthwash.

Did you feel any of the following discomfort while using mouthwash? (GRAPH 12)

Most of the participants felt no discomfort while using mouthwash .Some of the participants felt taste alteration in chlorhexidine mouthwash.

How was the taste of mouthwash (GRAPH 13)

In all the groups, felt pleasant mouthwash except plant extract and distilled water mouthwash felt unpleasant.

Did you feel any of the following discomfort in general health while using mouthwash? (GRAPH 14)

Most of the participants didn’t felt any discomfort in general health while using mouthwash.

Table 3 : Age distribution of the study participants in four groups

Group	Gender			
	Male		Female	
	n	%	n	%
GROUP A	10	50.0	10	50.0
GROUP B	10	50.0	10	50.0
GROUP C	10	50.0	10	50.0
GROUP D	10	50.0	10	50.0
'p'	1.0 Not Significant			

***Chi square test** ****p>0.05**

Table 4 : Inter group comparison of Mutans Streptococci Count (CFU/ml) values between the four groups before using mouthwash

Group	Mutans Streptococcui Count (CFU/ml) values before mouthwash (in millions)	
	Mean	**S.D.**
GROUP A	9.33	2.25
GROUP B	8.37	1.64
GROUP C	8.63	0.55
GROUP D	9.11	1.26
'p' value between Groups		
All the four Groups	0.195 Not Significant	
GROUP A & GROUP B	0.132 Not Significant	
GROUP A & GROUP C	0.186 Not Significant	
GROUP A & GROUP D	0.712 Not significant	
GROUP B& GROUP C	0.504 Not Significant	
GROUP B & GROUP D	0.115 Not Significant	
GROUP C& GROUP D	0.124 Not Significant	

*** p > 0.05**

Table 5 : Inter group comparison of Mutans Streptococci Count (CFU/ml) values between the four groups after using nouthwash

Group	Mutans Streptococci count after mouthwash (in millions)	
	Mean	**S.D.**
GROUP A	2.99	0.58
GROUP B	4.1	0.82
GROUP C	6.97	0.71
GROUP D	2.83	0.68
'p' value between Groups		
All the four Groups	**< 0.001 Significant**	
GROUP A & GROUP B	**< 0.001 Significant**	
GROUP A & GROUP C	**< 0.001 Significant**	
GROUP A & GROUP D	0.436 Not significant	
GROUP B& GROUP C	**< 0.001 Significant**	
GROUP B & GROUP D	**< 0.001 Significant**	
GROUP C& GROUP D	**< 0.001 Significant**	

*** p > 0.05**

Table 6 : Inter group comparison of change in Mutans Streptococci Count (CFU/ml) values between the four groups before and after using mouthwash

Group	Change in Mutans Streptococci count before and after mouthwash (in millions)	
	Mean	**S.D.**
GROUP A	6.34	2.51
GROUP B	4.27	1.15
GROUP C	1.66	0.62
GROUP D	6.28	1.15
'p' value between Groups		
All the four Groups	**< 0.001 Significant**	
GROUP A & GROUP B	**0.002 Significant**	
GROUP A & GROUP C	**< 0.001 Significant**	
GROUP A & GROUP D	0.927 Not significant	
GROUP B& GROUP C	**< 0.001 Significant**	
GROUP B & GROUP D	**< 0.001 Significant**	
GROUP C& GROUP D	**< 0.001 Significant**	

*** p > 0.05**

Table 7 :Changes within Plant extract + Methanol Group

Values in Plant extract + Methanol Group	Mutans Streptococci count (in millions)	
	Mean	**S.D.**
Before mouthwash	9.33	2.25
After mouthwash	2.99	0.58
Changes after mouthwash	6.34	2.51
% of changes after mouthwash	64.8	16.6
'p' value between before & after mouthwash	**< 0.001 Significant**	

*** p > 0.05**

Table 8 :Changes within Methanol Group

Values in Methanol Groups Group	Mutans Streptococci count (in millions)	
	Mean	**S.D.**
Before mouthwash	8.37	1.64
After mouthwash	4.1	0.82
Changes after mouthwash	4.27	1.15
% of changes after mouthwash	50.6	7.0
'p' value between before & after mouthwash	**< 0.001 Significant**	

*** p > 0.05**

Table 9: Changes within Plant extract + distilled water Group

Values in Plant extract + distilled water Group	Mutans Streptococci count (in millions)	
	Mean	**S.D.**
Before mouthwash	8.63	0.55
After mouthwash	6.97	0.71
Changes after mouthwash	1.66	0.62
% of changes after mouthwash	19.2	7.1
‘ p’ value between before & after mouthwash	**< 0.001 Significant**	

*** p > 0.05**

Table 10 : Changes within Chlorhexidine Group

Values in Chlorhexidine Group	Mutans Streptococci count (in millions)	
	Mean	**S.D.**
Before mouthwash	9.11	1.26
After mouthwash	2.83	0.68
Changes after mouthwash	6.28	1.15
% of changes after mouthwash	68.7	6.8
'p' value between before & after mouthwash	**< 0.001 Significant***	

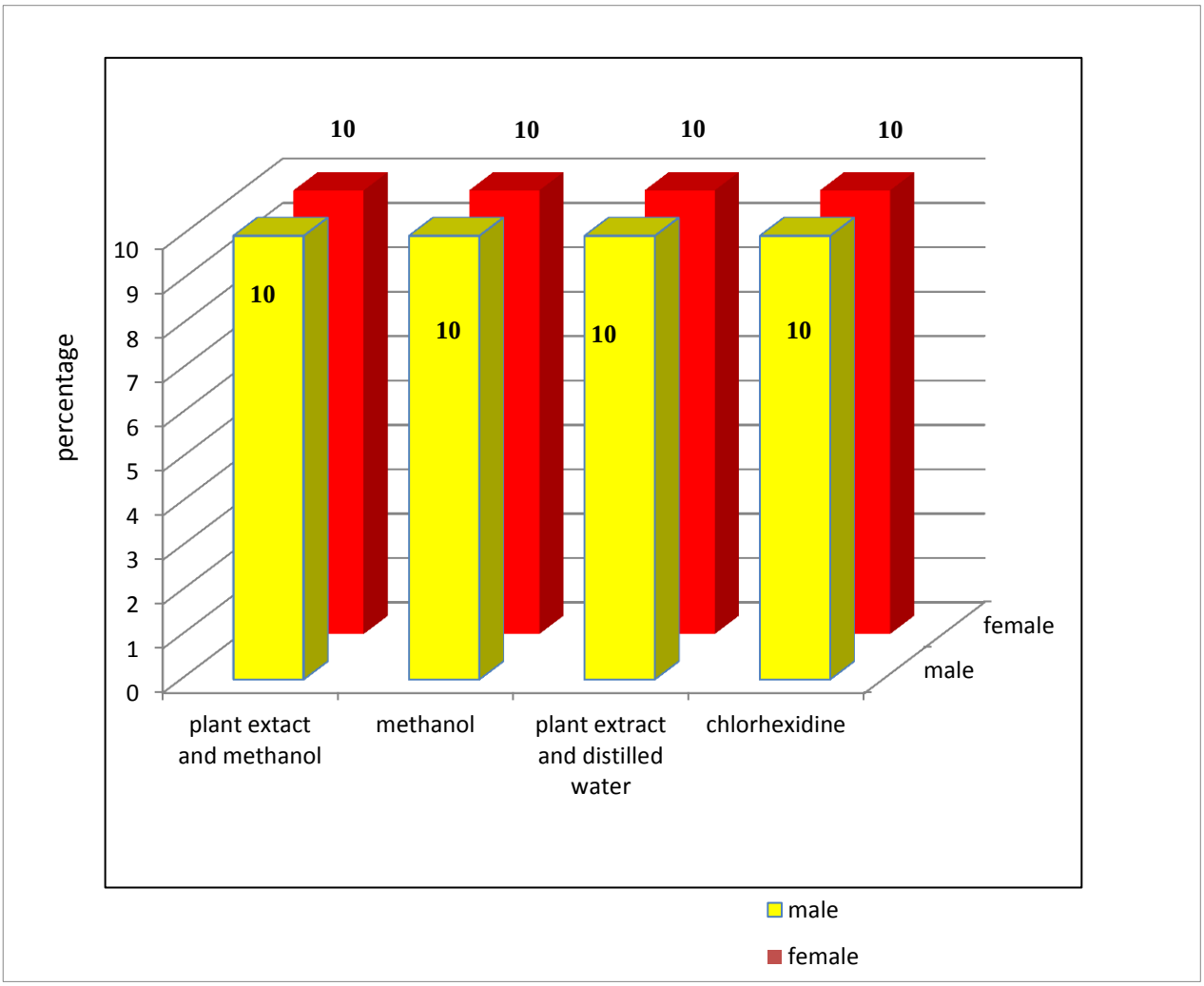
*** p > 0.05**

Table 11: Inter group comparison of percentage of change in Mutans Streptococci Count (CFU/ml) values between the four groups before and after using mouthwash

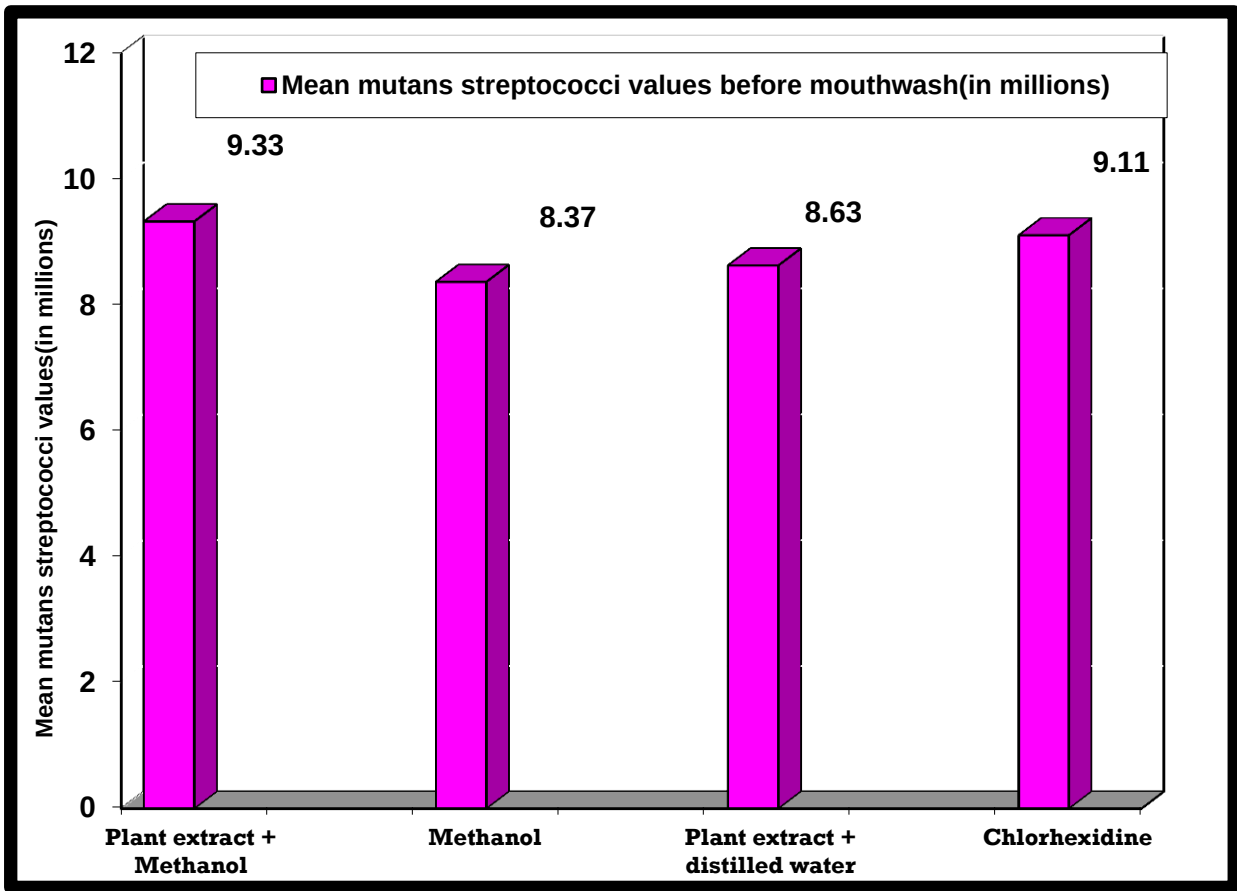
Group	% of Changes in Mutans Streptococci count before and after mouthwash	
	Mean	**S.D.**
GROUP A	64.8	16.6
GROUP B	50.6	7.0
GROUP C	19.2	7.1
GROUP D	68.7	6.8
'p' value between Groups		
All the four Groups	**< 0.001 Significant**	
GROUP A & GROUP B	**< 0.001 Significant**	
GROUP A & GROUP C	**< 0.001 Significant**	
GROUP A & GROUP D	0.333 Not significant	
GROUP B& GROUP C	**< 0.001 Significant**	
GROUP B & GROUP D	**< 0.001 Significant**	
GROUP C& GROUP D	**< 0.001 Significant**	

*** p > 0.05**

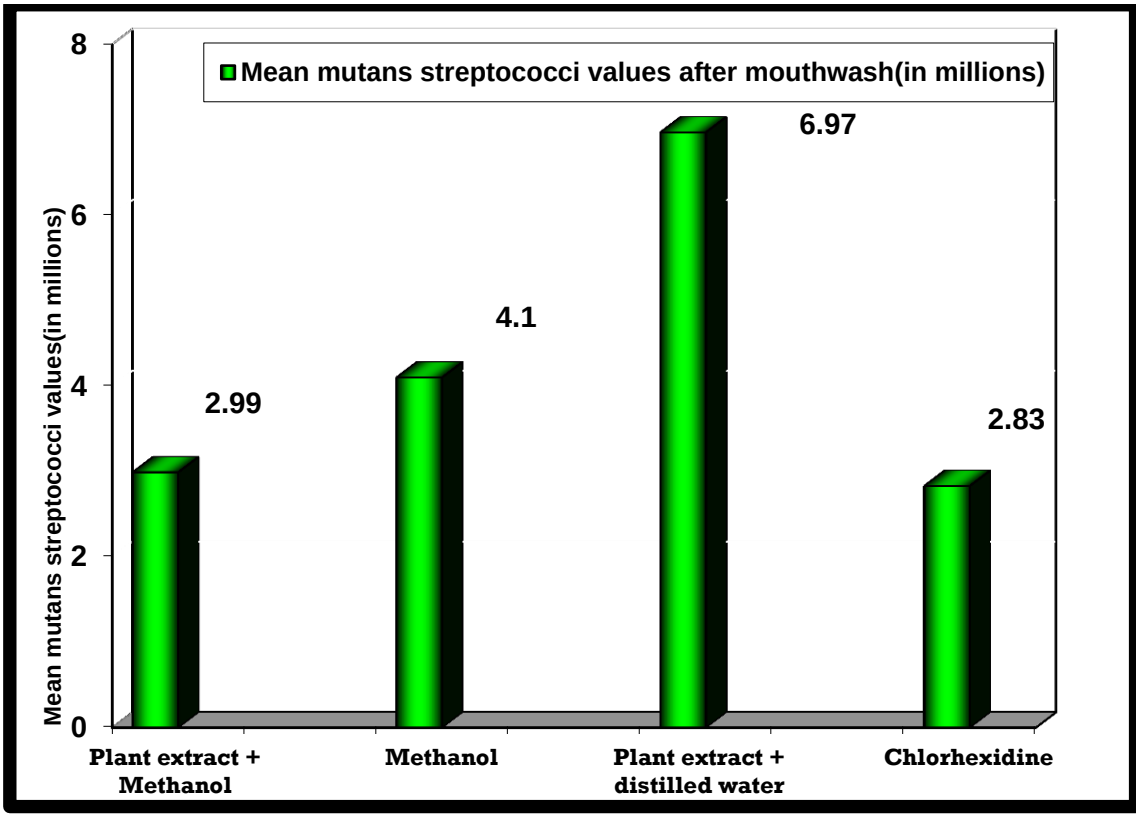
Graph 1 : Age distribution of the study participants in four groups



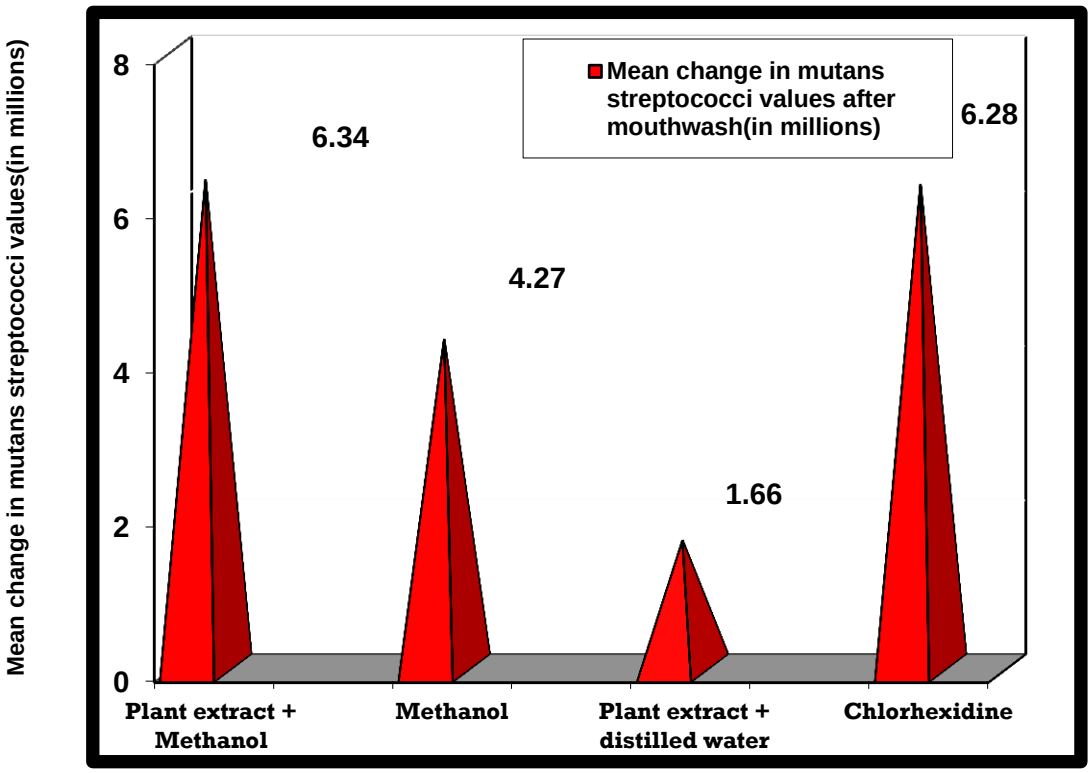
Graph 2: Mean Mutans Streptococci Count (CFU/ml) values before mouthwash



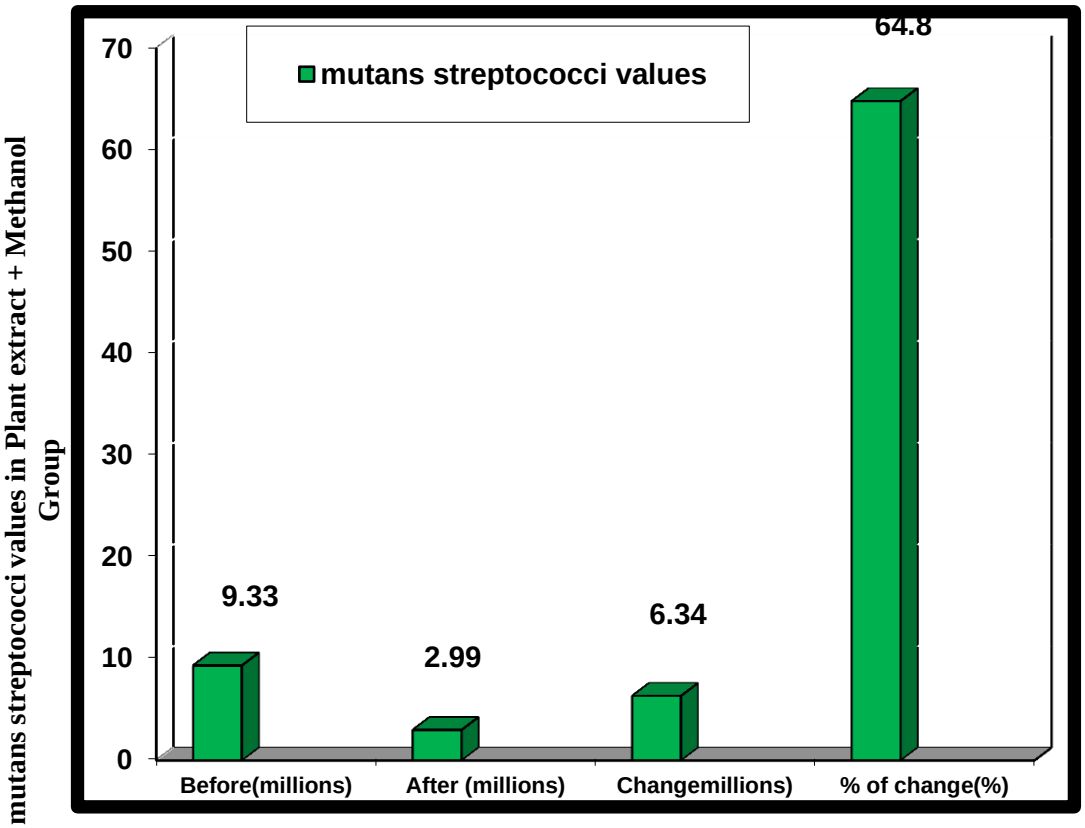
Graph 3: Mean Mutans Streptococci Count (CFU/ml) values after mouthwash



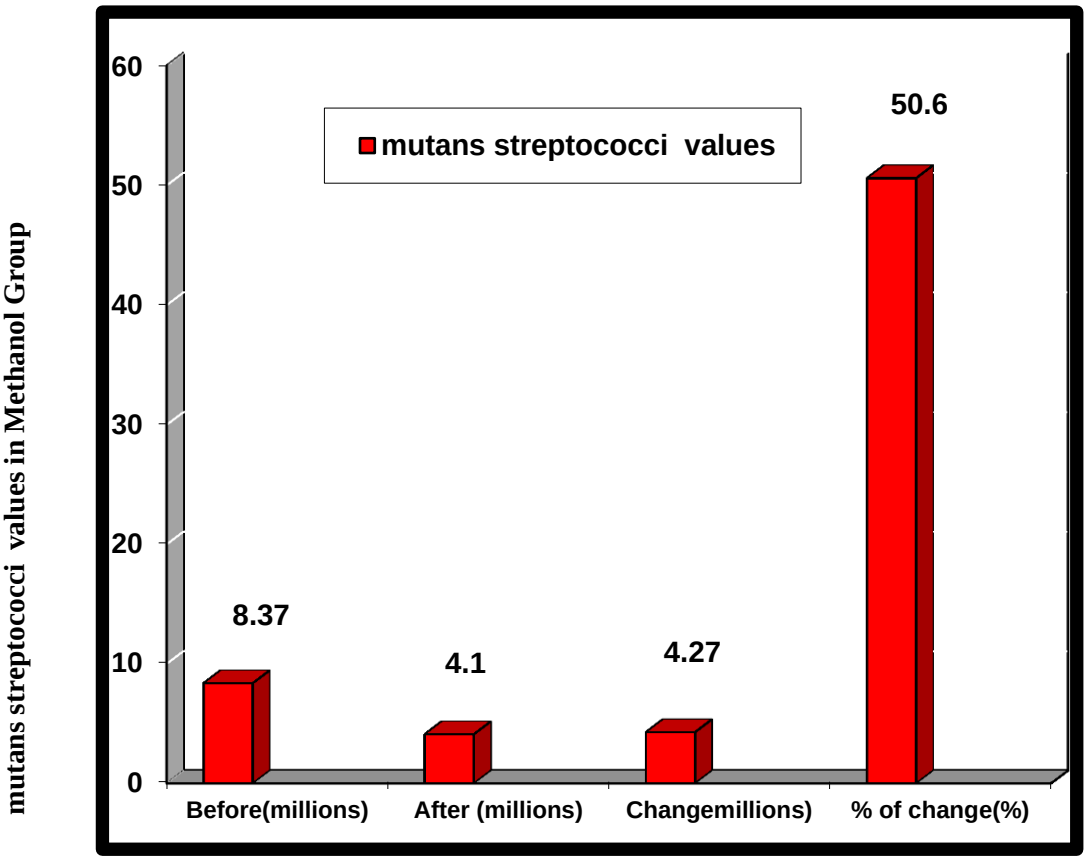
Graph 4: Mean change in Mutans Streptococci Count (CFU/ml) values after mouthwash



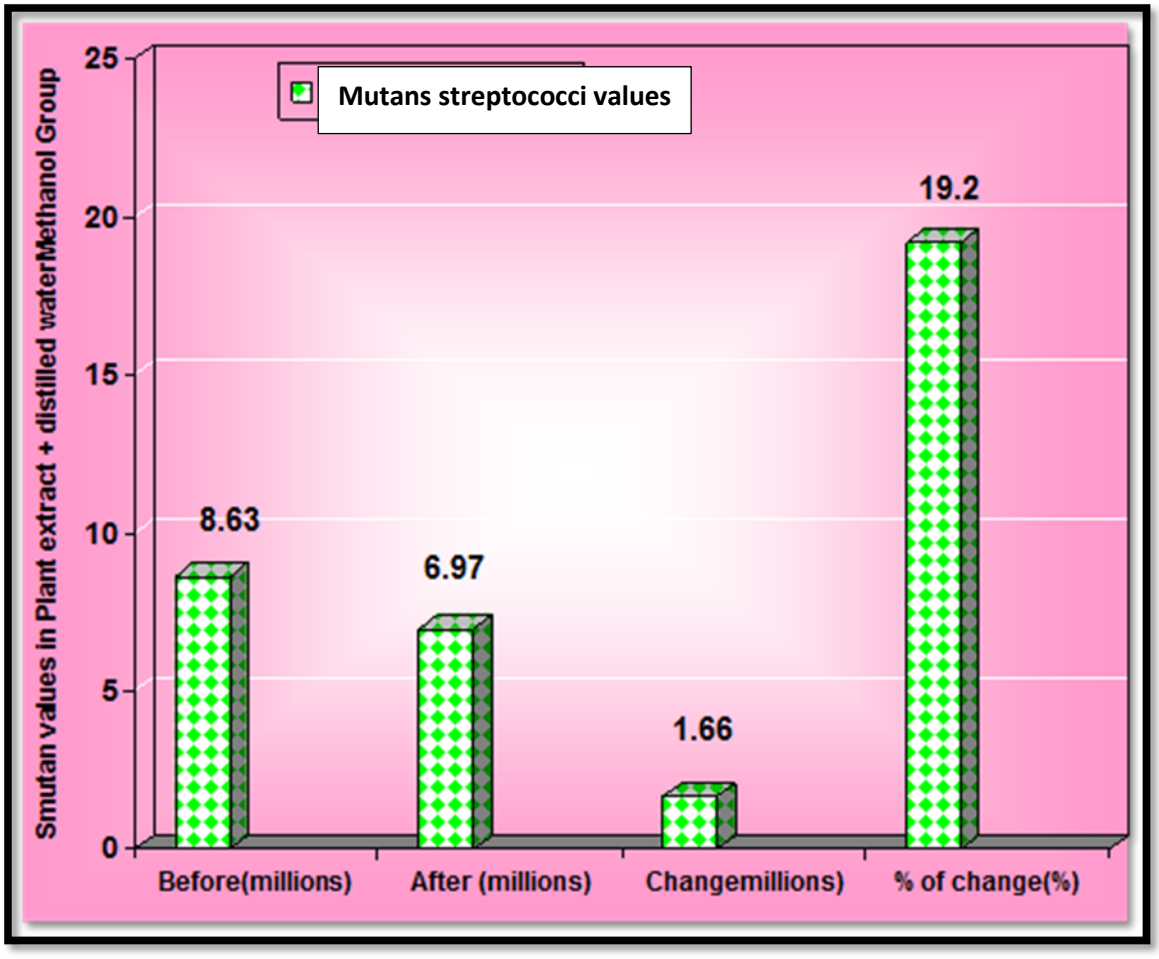
Graph 5: Change in Mutans Streptococci Count (CFU/ml) values in plant extract + methanol group after mouthwash



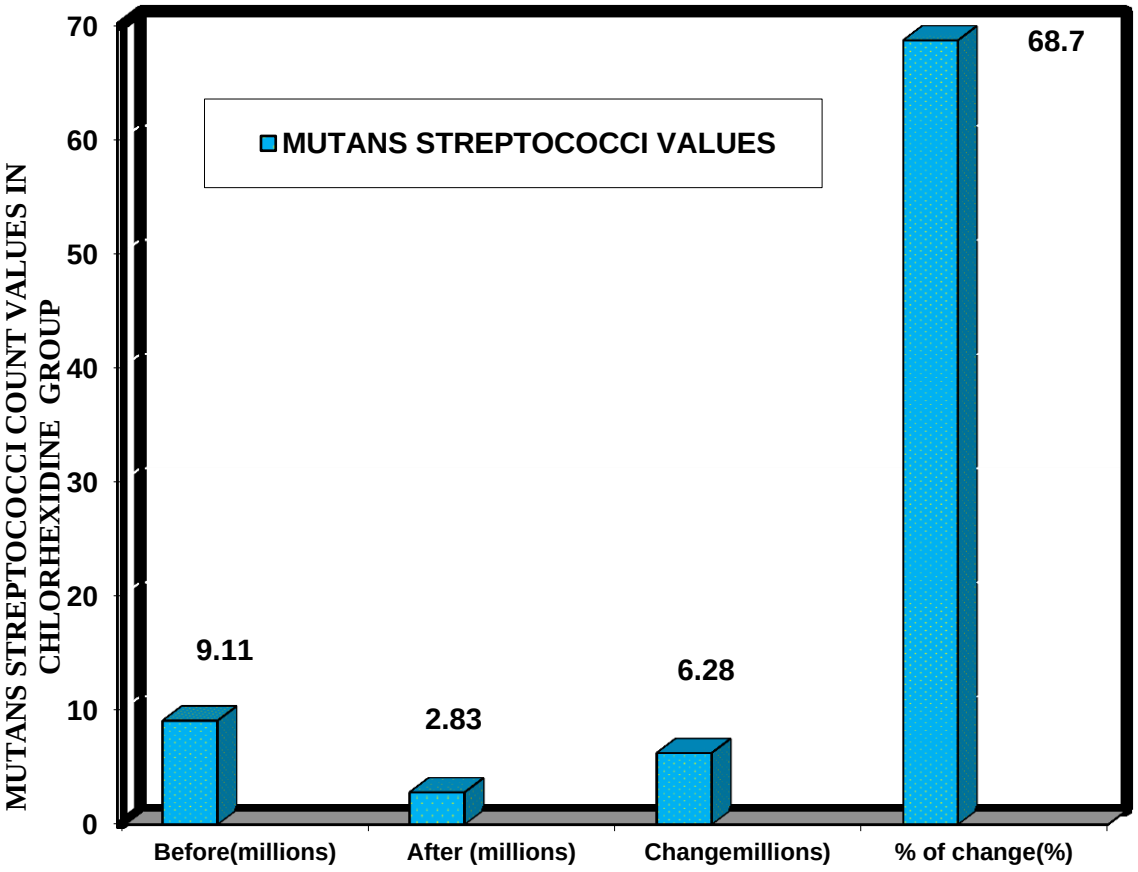
Graph 6: Change in Mutans Streptococci Count (CFU/ml) values in methanol group after using mouthwash



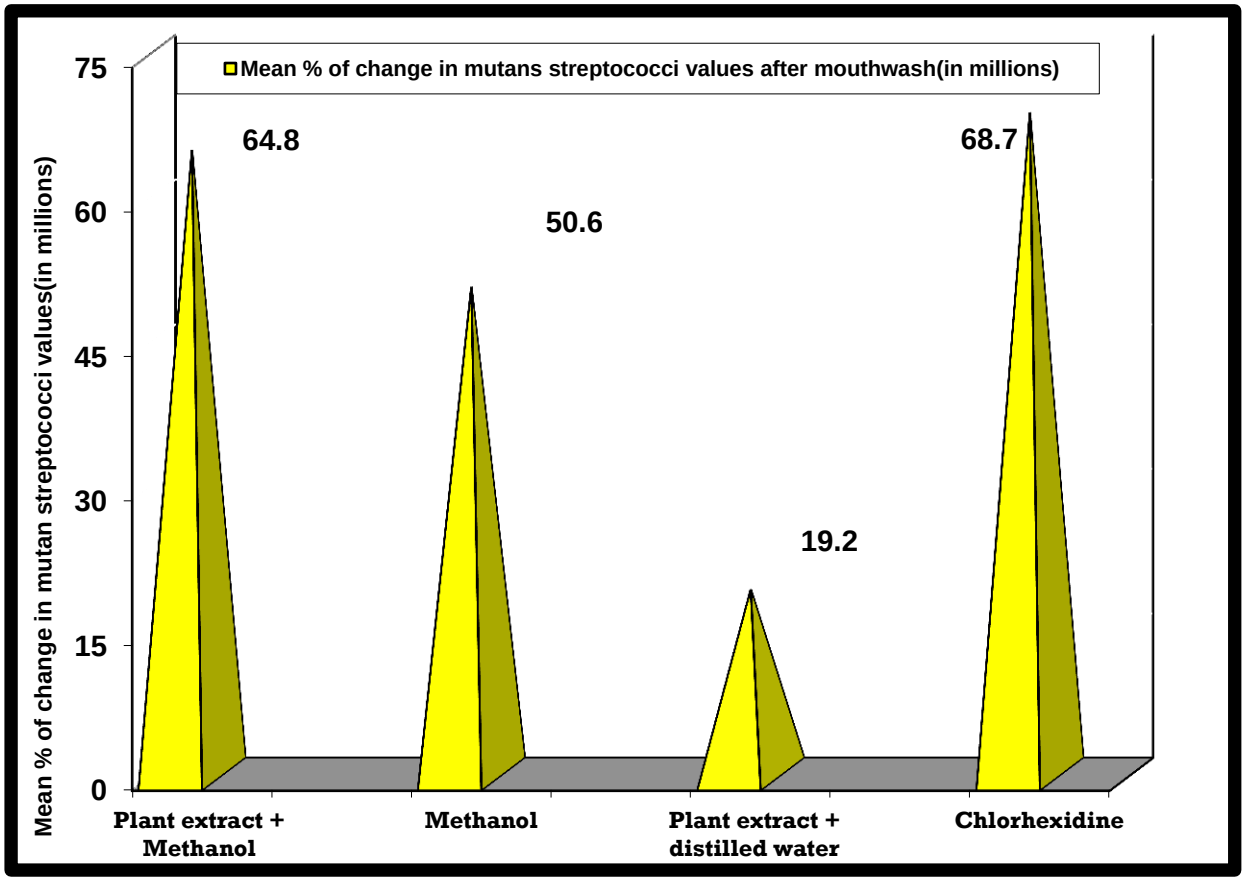
Graph 7: Change in Mutans Streptococci Count (CFU/ml) in plant extract + distilled water group after mouthwash



Graph 8: Change in Mutans Streptococci Count (CFU/ml) values in chlorhexidine group after mouthwash



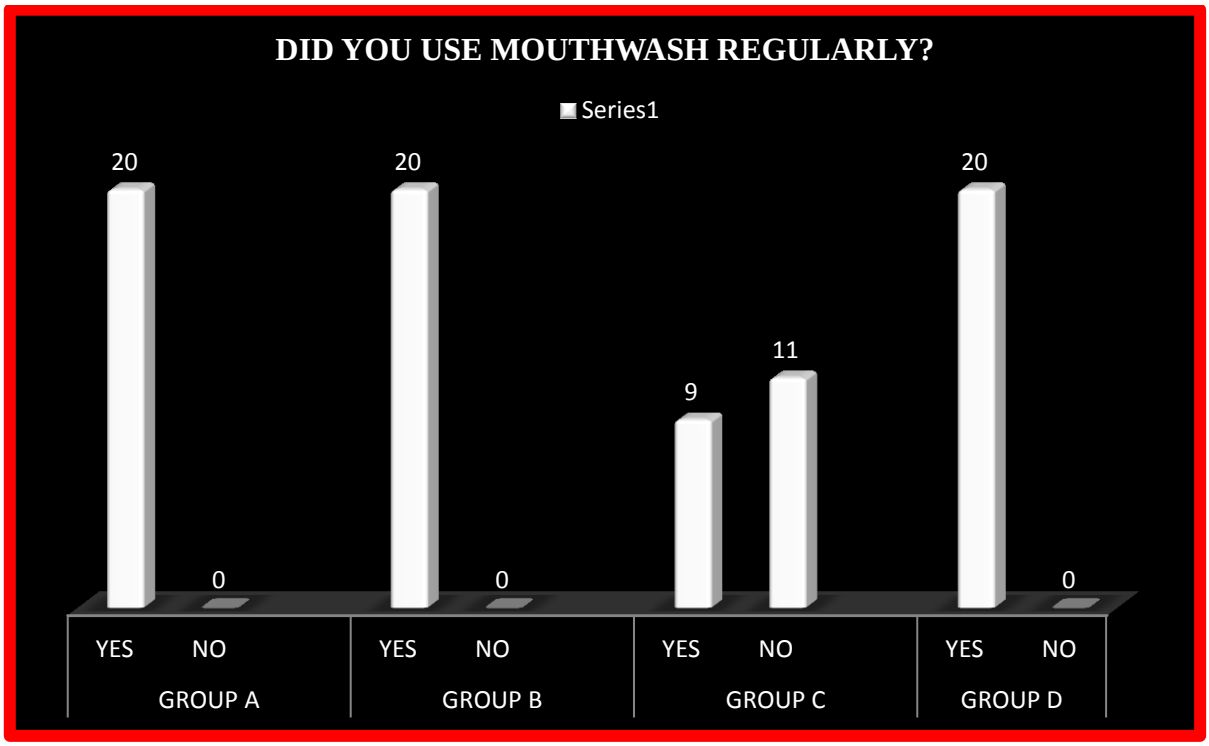
Graph 9: Mean % of change in Mutans Streptococci Count (CFU/ml) values after mouthwash



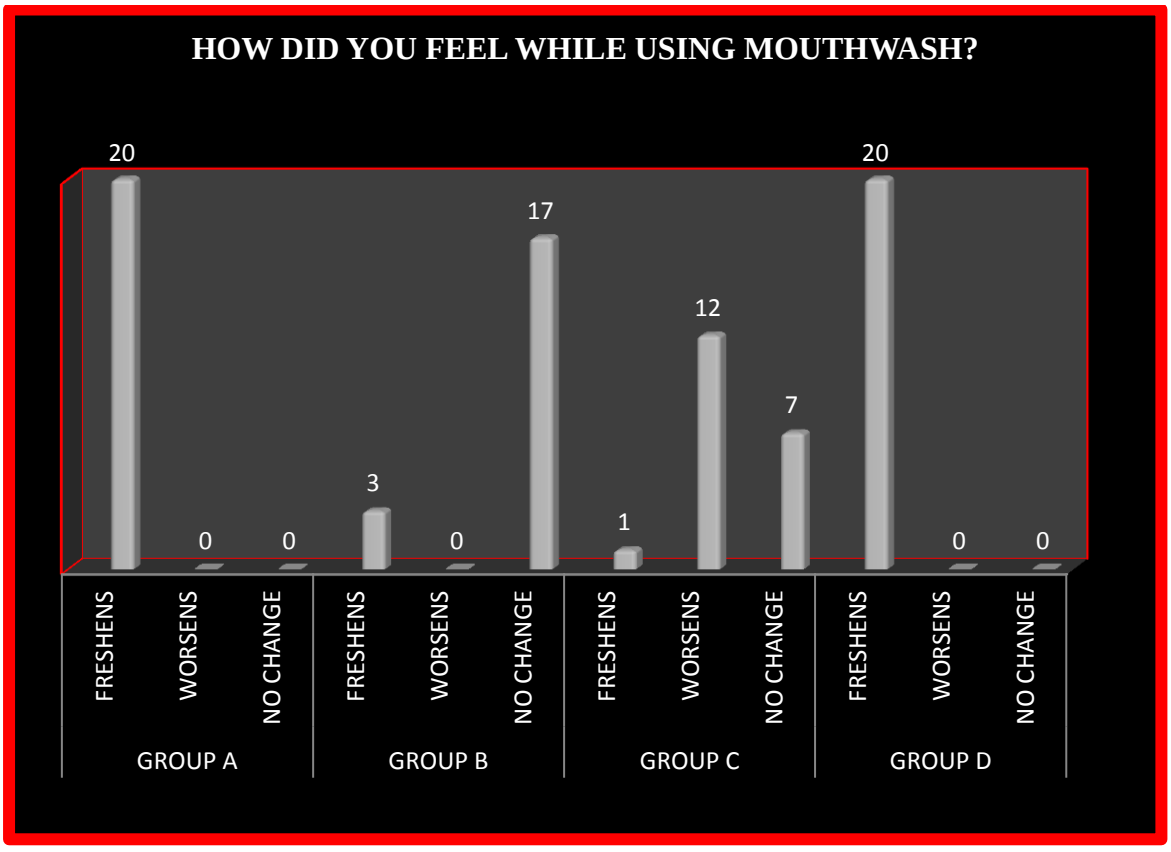
FEEDBACK RESULTS

FEEDBACK RESULTS

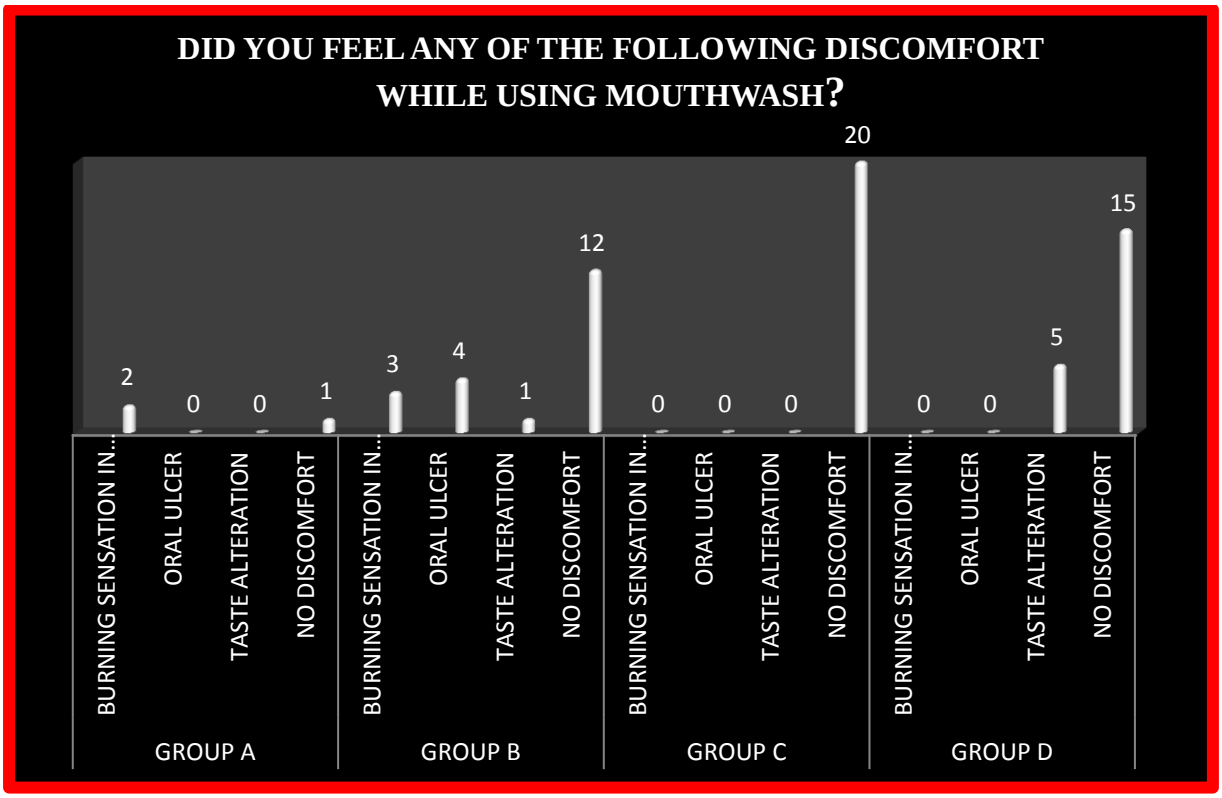
Graph 10: Did you use mouthwash regularly?



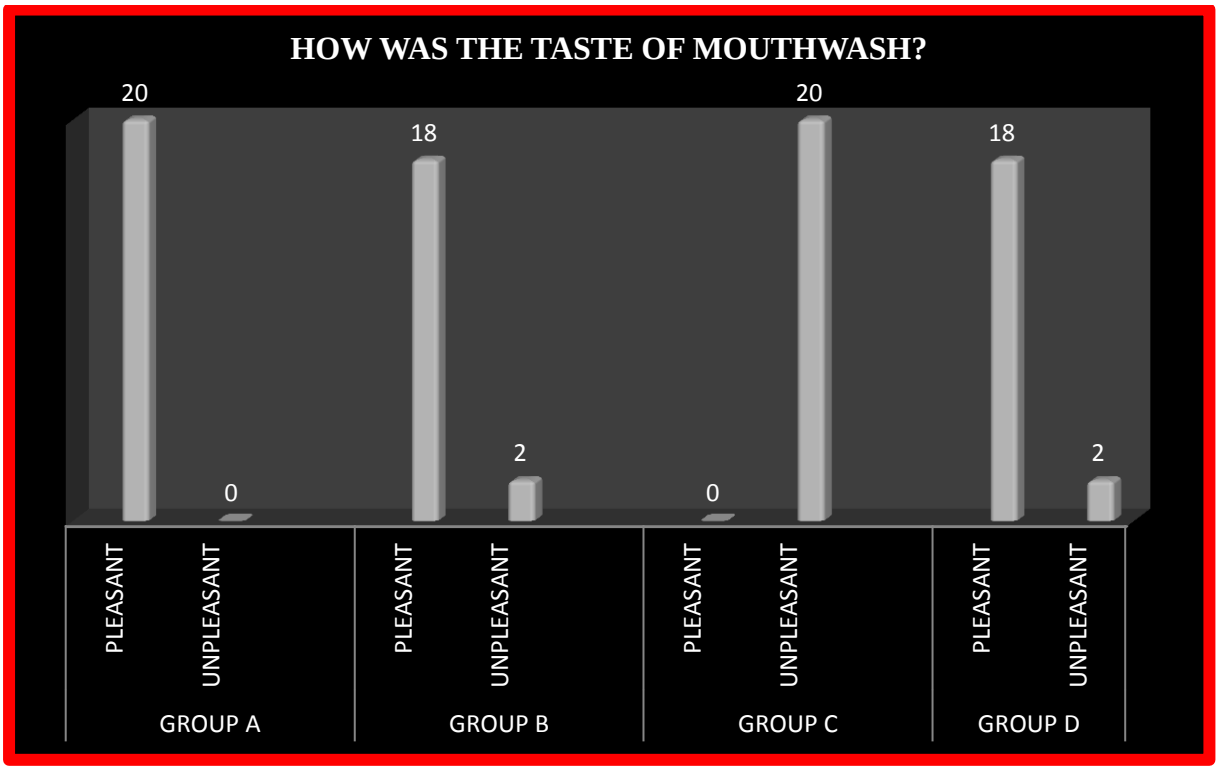
Graph 11: How did you feel while using mouthwash?



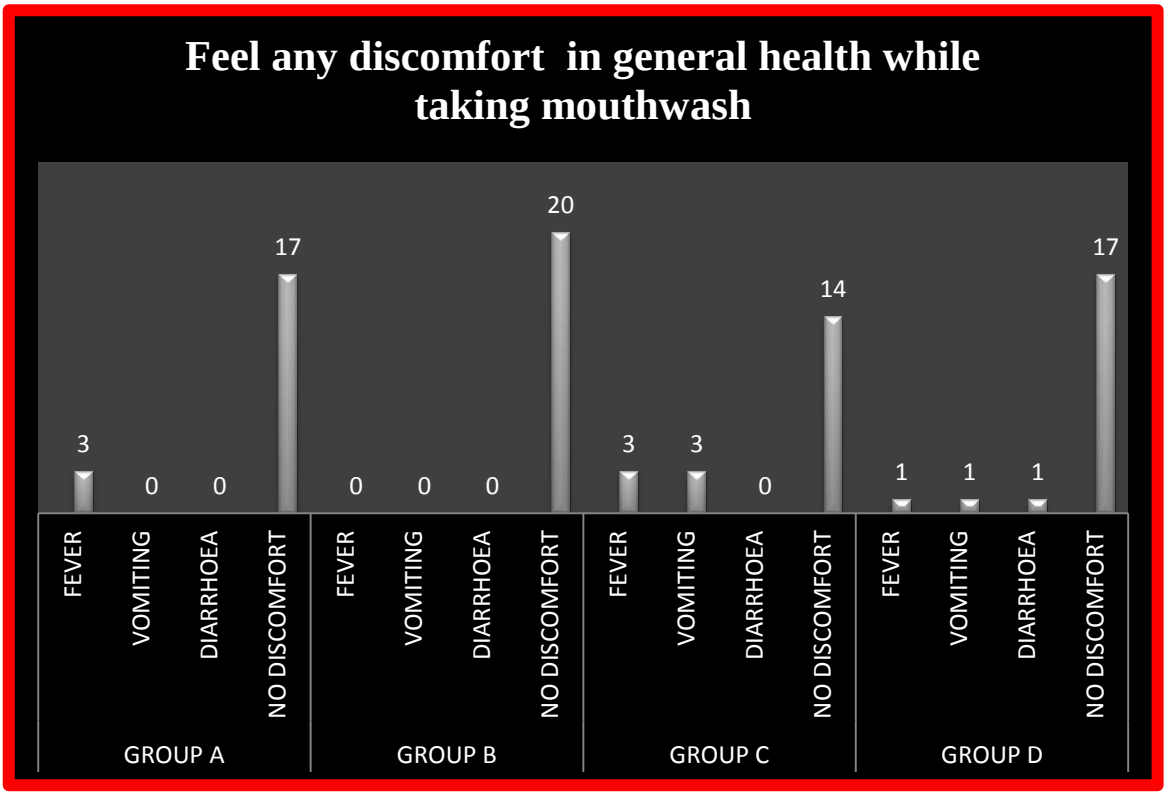
Graph 12: Did you feel any discomfort while using mouthwash?



Graph 13: Taste of mouthwash in all groups



Graph 14: Discomfort in general health while using mouthwash



DISCUSSION

DISCUSSION

Dental caries is claimed to be an ubiquitous and almost universal bacterial infection that has been afflicting mankind since the days of civilization. It is also hailed as an expensive disease, owing to the cost in terms of money spent for treatment as well as the human suffering it wroughts. Ever since Keyes in 1969, stated that the caries is produced by interaction between the factors such as tooth, oral microflora and the dietary substrates, it deemed fit to call dental caries as a "multifactorial disease".[10]

The pathogenesis of caries involves the synthesis of sticky glucans by the oral microbes, adherence of bacteria to the hard tissues and formation of plaque, and the acid production at the tooth-plaque interface. [10]Theoretically speaking, inhibition of each of these steps leads to the prevention of dental caries. Mechanical plaque control is said to be the best approach to prevent biofilm formation and chemotherapeutic agents can be used as an effective adjunct for this purpose. [11]

Miller's chemicoparasitic theory incriminated dental plaque to be a causative factor of dental caries in the early 20th century. Researchers had already started a laborious search for the specific elusive cause of dental caries and pointed out to Mutans Streptococci in the oral cavity as the primary causative microorganism of dental caries. Since then, various antimicrobial agents have been tried and tested against these micro-organisms. Some of them entered the markets while others remain at the research level.

Chlorhexidine is considered as the "gold standard" antimicrobial agent as it exhibits bacteriostatic properties at low concentrations and bactericidal properties at higher concentrations. It is used as a positive control in several studies that explore antibacterial agents. However longterm use of Chlorhexidine is not advocated due to the side-effects like taste alterations, dental staining and mucosal erosions.[3]

Awareness about the side effects of chemical plaque control agents have brought to light several herbal products that have antibacterial properties. Some of them include, Turmeric (Curcuma longa), Triphala, Guava (Psidium guajava), Garlic (Allium sativum), Neem (Azadirachta indica), Lime (Citrus aurantifolia), Pomegranate (Punica granatum), Green tea (Camellia sinensis), Tulsi (Ocimum sanctum), Cranberry (Vaccinium macrocarpon) etc.[12] the present research introduced and explored medicinal herbal plant Spilanthes acmella.

The leaves and flower heads of Spilanthes acmella have analgesic, antifungal, antihelminthic, antibacterial immunomodulatory, adaptogenic, lithotriptic, antiscorbitic, sialagogine, local astringency and digestive property. The plant also has vasorelaxant and antioxidant and local anesthetic and antipyretic activities due to presence of spilanthol which have been substantiated by lab and animal studies.[5] It is necessary to explore the phytochemical constituents of any medicinal plant to establish a relation between pharmacology and chemistry of the plant. The major pungent constituent reported in this plant S.acemella is "spilanthol," Triterpenoids have also been found in the plant. Spilanthol is chemically N isobutylamide which is bitter in taste and could stimulate salivation.[8]

An extensive literature search shows that there is an antibacterial property of Spilanthes acmella in in vitro research and there is no in vivo research carried out on health and oral health. Considering easy availability of plants in tropical country, herbal alternative as an antimicrobial and other medicinal properties. A new step as an in vivo research has been planned and conducted to assess antibacterial efficacy of Spilanthes acmella on salivary Mutans Streptococci with the confidence interval 95%, power of the study 90%. Assessment of minimum inhibitory concentration, toxicity, shelf life, palatability, Standardization of dose has been done well in prior.

SALIVARY PARAMETERS

Reasons for choosing mutans streptococci count in saliva as the primary outcome

Mutans Streptococci are the surrogate marker for dental caries as they are implicated to be the initiators of the disease and their quantity in the saliva is directly related to the number of surfaces colonized by them. Any intervention that can hamper their growth and survival will negatively impact the initiation and progress of caries. Mutans Streptococci also play an important role in caries prediction. Individuals who are heavily colonized by these microbes are classified to be at high risk for caries. They can adhere to the oral hard tissues by producing sticky dextrans by glucosyl transferase activity on sucrose.[13]

Reasons for choosing stimulated whole saliva

The participant chews the rubber band to transfer the bacteria from the tooth surfaces into the saliva. Whole saliva is a representative sample of not just the secretions from the major and minor glands but also microbes and their products, gingival exudates, epithelial cells and food debris. Whole saliva is said to be of clinical relevance for caries susceptibility and carious activity. With respect to stimulation, there is a huge increase in terms of salivary output, the consistency and the concentration of many of its ingredient such as proteins, immunoglobulins etc.[14]

INTERVENTIONAL MOUTHWASH

Group A: Spilanthes acmella plant extract + Methanol mouthwash.

There is a maximum changes and p value is statistically significant difference between before and after using Spilanthes acmella plant extract + methanol mouthwash. 64.8% reduction is seen after using mouthwash. Hence, this shows antibacterial activity of this mouthwash on salivary Mutans Streptococci with no adverse effects. The study participants feel freshens, no discomfort in general and oral health when regular use of mouthwash. Palatability of this mouthwash is pleasant and good. This is due to the presence of phytochemicals present in the plant such as spilanthol and triterpenoids. This result is in positive agreement with other outstanding previous study proves that Triphala has an inhibitory effect on Streptococcus mutans and Lactobacilli.[17] Other herbal studies on Turmeric, Guava, Garlic, Neem, Lime, Pomegranate, Green tea, Tulsi, Cranberry etc. show antibacterial property with less side effects and cost effective oral hygiene products.

Group B : Methanol mouthwash

p value is statistically significant difference between before and after using 5% methanol mouthwash. 50% reduction is seen after using methanol mouthwash. The study participants feel freshens, no discomfort in general and oral health when regular use of mouthwash. Palatability of this mouthwash is pleasant and good. Hence, methanol has its own antibacterial property due to the presence of phenol compound. This leads to decomposition of the membrane of microbes.[18]

Group C: Spilanthes acmella plant extract + distilled water mouthwash

There is minimum changes and p value is statistically significant between before and after using Spilanthes acmella plant extract + distilled water mouthwash. Minimal percentage reduction is seen after using this mouthwash. The study participants feel poor palatability, unpleasant taste, half of the participants refused to use this mouthwash regularly. But there is no discomfort in general and oral health. The present study proves that presence of phytochemicals such as spilanthol and triterpenoids in this plant shows antibacterial activity on salivary Mutans Streptococci. This result is in positive agreement with other outstanding previous study proves that Garlic has an antibacterial efficacy due to the presence of allicin, most therapeutically potent on oral salivary microorganisms.[19]

Group D: Chlorhexidine mouthwash.

There is a maximum changes and p value is statistically significance between before and after using chlorhexidine mouthwash. 68.7% reduction was seen after using this mouthwash. Chlorhexidine mouthwash as gold standard, already proved that antibacterial efficacy of this mouthwash on salivary Mutans Streptococci.[18] The study participants feel freshens, no discomfort in general and oral health when regular use of mouthwash.

Palatability of this available mouthwash is pleasant and good. This result is in positive agreement with other outstanding previous study proves that chlorhexidine mouthwash shows antibacterial efficacy.[19]

Comparison between groups before intervention

At baseline

There is no statistically significant differences seen before intervention.

Comparison between groups after intervention

i) Group A and Group B

When comparing between Group A and Group B, there is statistically significant difference seen after intervention. Maximum mean reduction seen in Group A. This says that Spilanthes acmella plant extract + methanol mouthwash shows efficacious than methanol mouthwash. Reason may be due to the synergistic action of Spilanthes acmella plant extract + methanol mouthwash shows antibacterial efficacy on salivary mutans streptococci than the plain 5% methanol. Due to the presence of phytochemicals such as spilanthol and triterpenoids present in the plant and phenolic compounds in the alcohol. This result is in positive agreement with other outstanding previous study proves that methanolic extract of Guava shows an antibacterial efficacy against infections and disease caused by Gram positive bacteria.[20]

ii) Group A and Group C.

When comparing between Group A and Group C, there is statistically significant difference seen after intervention. There is a maximum mean reduction seen in Group A. This says that Spilanthes acmella + methanol mouthwash shows efficacious when comparing with Spilanthes acmella + distilled water mouthwash. Reason may be due to the synergistic action of spilanthol, triterpenoids and phenolic compounds shows antibacterial efficacy on mutans streptococci than irregular use of spilanthes acmella plant extract + distilled water mouthwash of participants feel as an unpalatable. This result is in positive agreement with other outstanding previous study proves that Tulsi has an antibacterial efficacy on periodontal pathogens.[21]

iii) Group A and Group D.

When comparing between Group A and Group D, there is no statistically significant difference seen after intervention. There is an equal mean reduction seen in Group A & Group D. This says that Spilanthes acmella plant extract + methanol mouthwash and chlorhexidine mouthwash shows equally efficacious. This shows that 20% Methanolic extract of Spilanthes acmella plant extract as better as gold standard chlorhexidine mouthwash. The positive agreement with previous study proves that Green tea and Chlorhexidine mouthwash shows equally efficacious in the management of dental plaque induced gingivitis.[22] Turmeric and chlorhexidine mouthwash shows equally efficacious in the prevention of plaque and gingivitis.[23]

iv) Group B and Group C.

When comparing Group B and Group C, there is statistically significant difference seen after intervention. There is slight mean reduction seen in Group B. This says that methanol mouthwash shows efficacious than Spilanthes acmella plant extract + distilled water mouthwash. Hence methanol has its own antibacterial property due to the presence of phenol compound. This leads to decomposition of the membrane of microbes.

v) Group B and Group D.

When comparing Group B and Group D, there is statistically significant difference seen after intervention. There is a maximum mean reduction seen in Group D. This says that chlorhexidine mouthwash shows efficacious than methanol mouthwash. Chlorhexidine as gold standard already proves that antibacterial efficacy on salivary Mutans streptococci. The positive agreement with previous study proves that pomegranate peel extract and chlorhexidine mouthwash shows antibacterial effect on streptococcus mutans in the oral cavity.[24]

v) Group C and Group D.

When comparing Group C and Group D, there is statistically significant difference seen after intervention. There is a maximum mean reduction seen in Group D. This says that chlorhexidine mouthwash shows efficacious than Spilanthes acmella plant extract + distilled water mouthwash. Reason is chlorhexidine as a gold standard mouthwash which shows more efficacious on salivary mutans streptococci than Spilanthes acmella plant extract + distilled water mouthwash. The positive agreement with previous study proves that Cranberry shows an antibacterial efficacy on streptococcal colonies.[25] Salvadora persica shows less antibacterial efficacy than chlorhexidine mouthwash in preventing dental plaque.[26]

Limitations of the study

The age group for the present study is narrow and the study results can only be extrapolated to this particular age group (15-17 years) and subjects who attend school educational institutions in Madurai and therefore the generalizability is limited. Only 20% Methanolic extract of Spilanthes acmella mouthwash is checked and tested only on mutans streptococci organism.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present research is a randomized, concurrent parallel arm, non invasive, interventional controlled trial designed to assess the ‘‘Anti-bacterial efficacy of Spilanthes acmella on salivary Mutans Streptococci.

The current research highlights the potential usage of Spilanthes acmella extract as an alternative and challenges that traditional belief of chlorhexidine as a gold standard.

The present research to test antibacterial of Spilanthes acmella on salivary Mutans Streptococci. The findings of the study underlines positive synergistic efficacy of methanol with Spilanthes acmella extract. It is interesting to note, this is the first in vivo study which explore the possible potency of Spilanthes acmella as a herbal mouthwash without the side effects of chlorhexidine.

This research also graces the clinical aspect of toxicity, shelf life, MIC, and palatability as a pharmacological medicated preparation.

To conclude as an author and a young budding researcher its been satisfactory to say that the antibacterial efficacy of 20% Methanolic extract of Spilanthes acmella plant has proven to be potent mouthwash without adverse effects expected with chlorhexidine. I call for further research and improvements in the current formulation and dispensing of this anti-toothache plant preparation.

RECOMMENDATIONS

RECOMMENDATIONS

This research aspired to explore the efficacy of Spilanthes acmella as a potential antibacterial mouthwash and it was concluded that 20% Spilanthes acmella plant extract was considerably efficacious in reducing the Mutans Streptococci count in comparison with the gold standard Chlorhexidine.

However further research might aid in strengthening this evidence established in the study.

- The present human research first to explore antibacterial property only on Mutans Streptococci in short period trials. Further research to explore in long term trial.
- This study herbal plants possess analgesic property. Further research to check the analgesic and periodontal pathogens related to oral health.
- Other results of the future research on Spilanthes acmella will combined and publishing as a review.
- Studies using Economic analysis to weigh the cost-effectiveness of herbal plant should also be done, to explore it as a public health intervention especially in a developing country like India.

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ANNEXURE 1

APPROVAL COPY OF STUDY PROTOCOL FROM HEAD OF THE DEPARTMENT AND GUIDE

SUBMITTED TO OFFICE

DATE : 29/12/2015

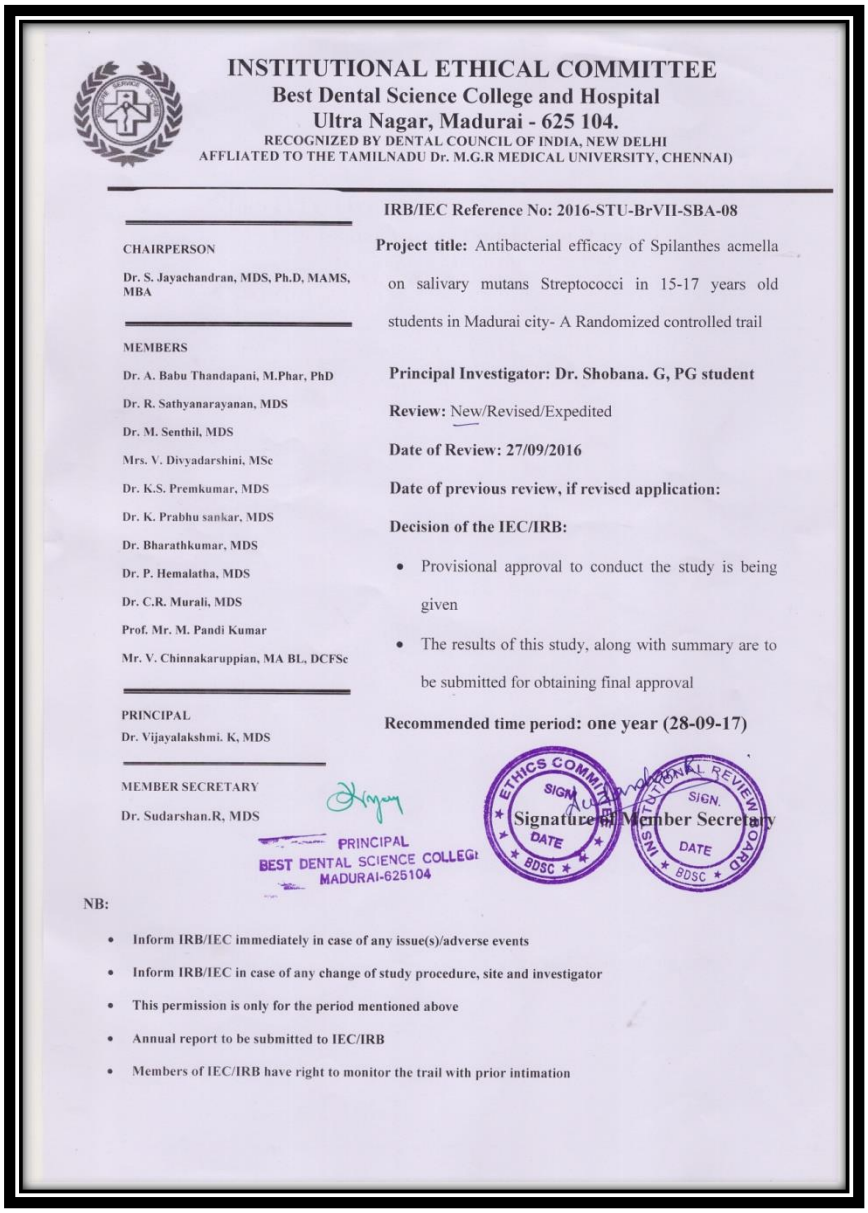
BEST DENTAL SCIENCE COLLEGE AND HOSPITAL

DEPARTMENT OF PUBLIC HEALTH DENTISTRY

1	TITLE OF THE STUDY	Antibacterial efficacy of *Spilanthes acmella* on salivary *Streptococcus mutans* in 15-17 years old students in Madurai city – A Randomized Controlled Trial (RCT)
2	NAME OF THE STUDENT	Dr.G. Shobana, First Year Post Graduate Student, Department of Public Health Dentistry.
3	NAME OF THE INSTITUTE	Best Dental Science College and Hospital, Madurai.
4	NAME AND DESIGNATION OF THE GUIDE	Dr.R.Muthu Karuppaiah, M.D.S., Reader, Department of Public Health Dentistry, Best Dental Science College and Hospital, Madurai.
5	SIGNATURE OF THE GUIDE	
6	SIGNATURE OF THE HEAD OF THE DEPARTMENT DR. BHARATH KUMAR GARLA M.D.S.,	PROFESSOR & H.O.D., DEPT. OF PUBLIC HEALTH DENTISTRY, BEST DENTAL SCIENCE COLLEGE, MADURAI-625104.

ANNEXURE 2

ETHICAL APPROVAL FOR THE RESEARCH FROM THE INSTITUTIONAL REVIEW BOARD



INSTITUTIONAL ETHICAL COMMITTEE
Best Dental Science College and Hospital
Ultra Nagar, Madurai - 625 104.
RECOGNIZED BY DENTAL COUNCIL OF INDIA, NEW DELHI
AFFLIATED TO THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY, CHENNAI)

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PRINCIPAL
Dr. Vijayalakshmi. K, MDS

MEMBER SECRETARY
Dr. Sudarshan.R, MDS

IRB/IEC Reference No: 2016-STU-BrVII-SBA-08

Project title: Antibacterial efficacy of Spilanthes acmella on salivary mutans Streptococci in 15-17 years old students in Madurai city- A Randomized controlled trail

Principal Investigator: Dr. Shobana. G, PG student

Review: New/Revised/Expedited

Date of Review: 27/09/2016

Date of previous review, if revised application:

Decision of the IEC/IRB:

- Provisional approval to conduct the study is being given
- The results of this study, along with summary are to be submitted for obtaining final approval

Recommended time period: one year (28-09-17)

PRINCIPAL
BEST DENTAL SCIENCE COLLEGE
MADURAI-625104

ETHICS COMMITTEE SIGN. DATE BDSC

INSTITUTIONAL REVIEW BOARD SIGN. DATE BDSC

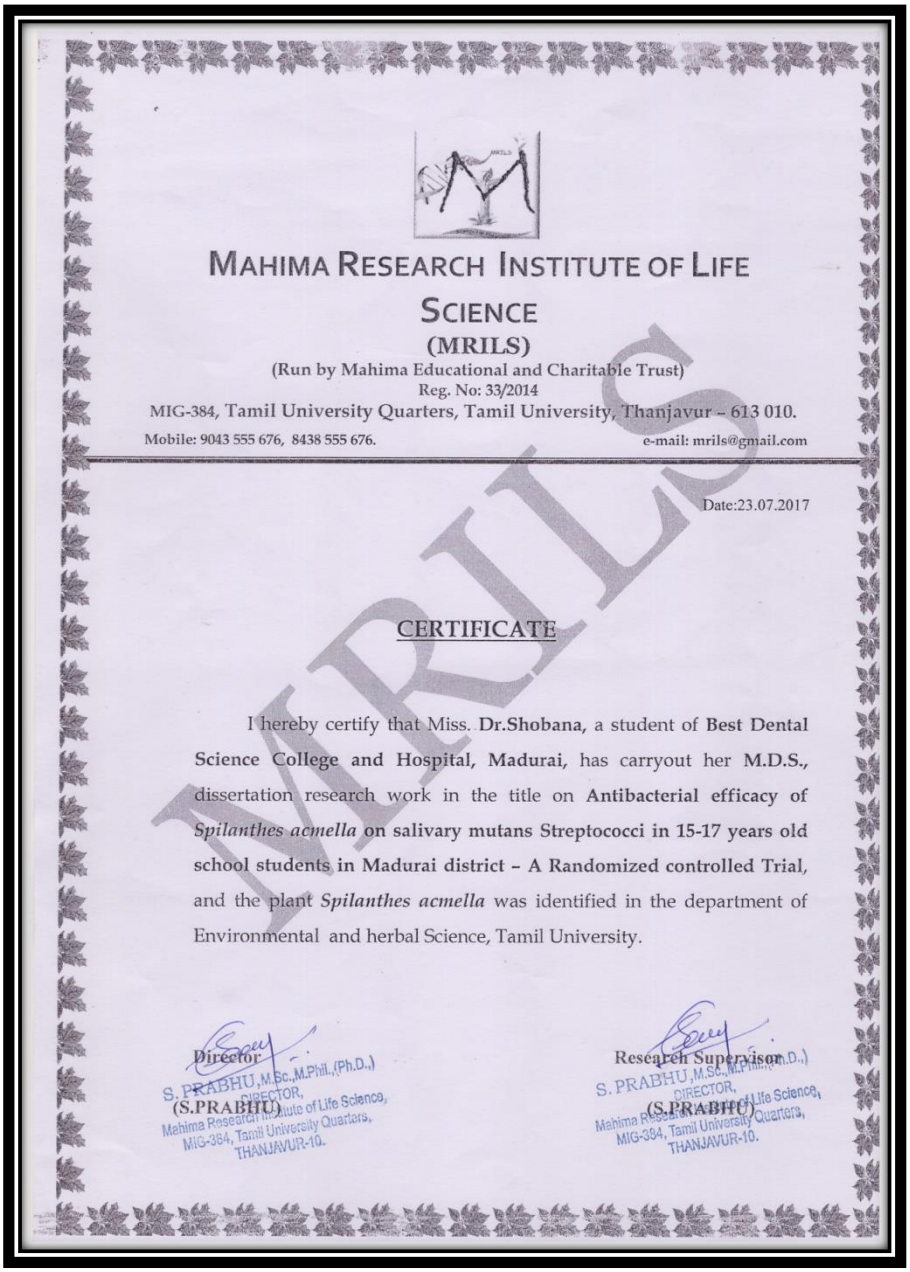
Signature of Member Secretary

NB:

- **Inform IRB/IEC immediately in case of any issue(s)/adverse events**
- **Inform IRB/IEC in case of any change of study procedure, site and investigator**
- **This permission is only for the period mentioned above**
- **Annual report to be submitted to IEC/IRB**
- **Members of IEC/IRB have right to monitor the trail with prior intimation**

ANNEXURE 3

CERTIFICATE FOR SPILANTHES ACMELLA HERBAL PLANT IDENTIFICATION FROM THE RESEARCH INSTITUTE



MAHIMA RESEARCH INSTITUTE OF LIFE SCIENCE
(MRILS)
(Run by Mahima Educational and Charitable Trust)
Reg. No: 33/2014
MIG-384, Tamil University Quarters, Tamil University, Thanjavur – 613 010.
Mobile: 9043 555 676, 8438 555 676. e-mail: mrils@gmail.com

Date:23.07.2017

CERTIFICATE

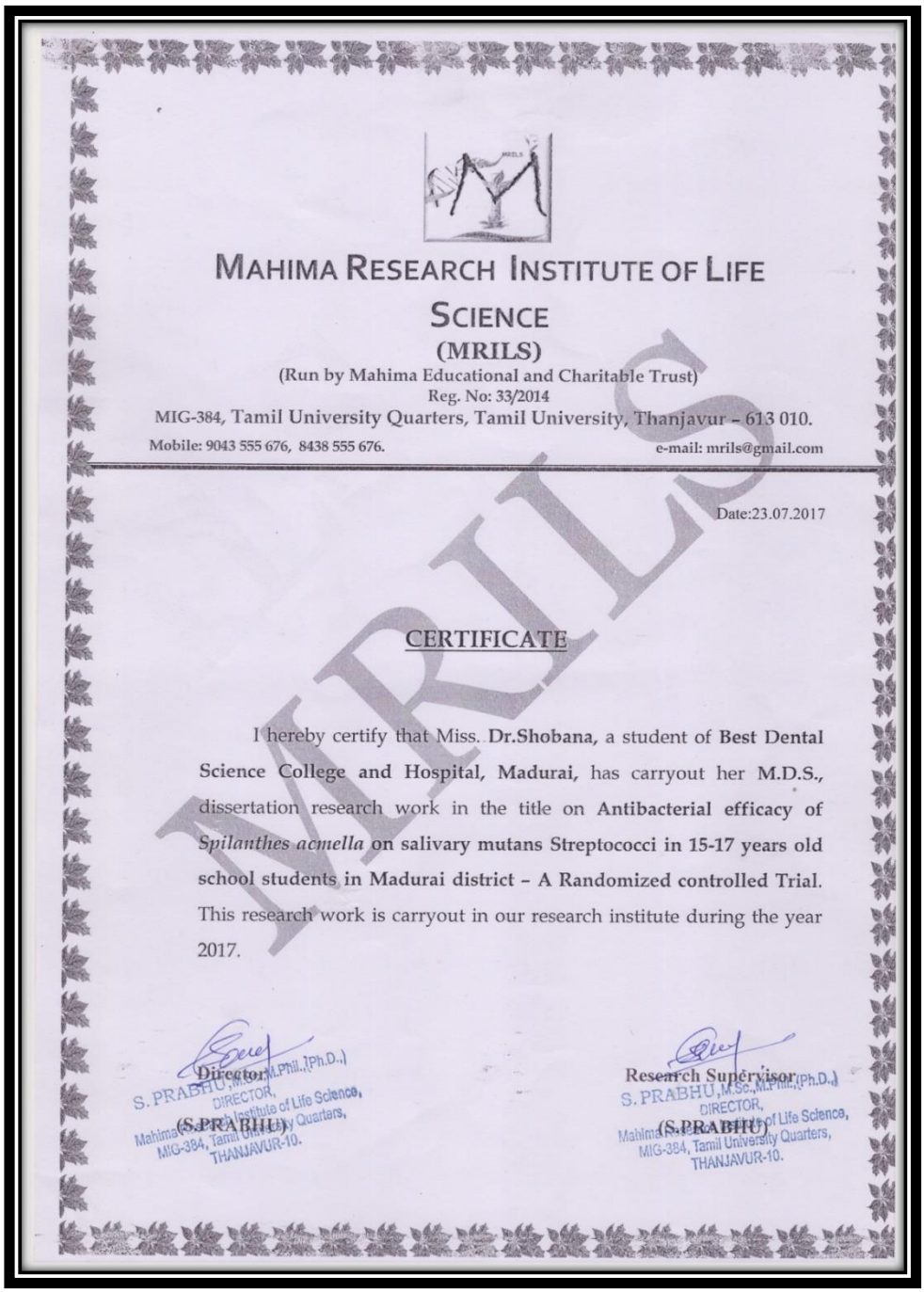
I hereby certify that Miss. **Dr.Shobana,** a student of **Best Dental Science College and Hospital, Madurai,** has carryout her **M.D.S.,** dissertation research work in the title on **Antibacterial efficacy of *Spilanthes acmella* on salivary mutans Streptococci in 15-17 years old school students in Madurai district – A Randomized controlled Trial,** and the plant ***Spilanthes acmella*** was identified in the department of Environmental and herbal Science, Tamil University.

Director
(S.PRABHU)
S. PRABHU, M.Sc., M.Phil., (Ph.D.,)
DIRECTOR,
Mahima Research Institute of Life Science,
MIG-384, Tamil University Quarters,
THANJAVUR-10.

Research Supervisor
(S.PRABHU)
S. PRABHU, M.Sc., M.Phil., (Ph.D.,)
DIRECTOR,
Mahima Research Institute of Life Science,
MIG-384, Tamil University Quarters,
THANJAVUR-10.

ANNEXURE 4

CERTIFICATE FOR PHARMACOLOGY (MOUTHWASH) PREPARATION FROM THE RESEARCH INSTITUTE



MAHIMA RESEARCH INSTITUTE OF LIFE SCIENCE
(MRILS)
(Run by Mahima Educational and Charitable Trust)
Reg. No: 33/2014
MIG-384, Tamil University Quarters, Tamil University, Thanjavur – 613 010.
Mobile: 9043 555 676, 8438 555 676. e-mail: mrils@gmail.com

Date:23.07.2017

CERTIFICATE

I hereby certify that Miss. **Dr.Shobana,** a student of **Best Dental Science College and Hospital, Madurai,** has carryout her **M.D.S.,** dissertation research work in the title on **Antibacterial efficacy of *Spilanthes acmella* on salivary mutans Streptococci in 15-17 years old school students in Madurai district – A Randomized controlled Trial.** This research work is carryout in our research institute during the year 2017.

Director
(S.PRABHU)
S. PRABHU, M.Sc., M.Phil., (Ph.D.,)
DIRECTOR,
Mahima Research Institute of Life Science,
MIG-384, Tamil University Quarters,
THANJAVUR-10.

Research Supervisor
(S.PRABHU)
S. PRABHU, M.Sc., M.Phil., (Ph.D.,)
DIRECTOR,
Mahima Research Institute of Life Science,
MIG-384, Tamil University Quarters,
THANJAVUR-10.

ANNEXURE 5

CERTIFICATE FOR DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

Research Training Consultancy

HARMAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH

Thanjavur, Tamil Nadu, South India.

Date:

CERTIFICATE

This is to certify that plant extract received from Dr. G. Shobana, studying final year P.G. Best Dental Science College and Hospital, Madurai, The extract tested against *in vitro* antibacterial activity against *Streptococcus mutans* in dose dependent manner. The results were represented in table 1.

Table 1 Antibacterial activity
(*Streptococcus mutans*)

Concentrations	50 µl	100 µl	150 µl	200 µl	Control (Methanol 30 µl)
1 %	Nil	Nil	Nil	Nil	Nil
3 %	Nil	Nil	Nil	0.50±0.03	Nil
5 %	Nil	Nil	Nil	2.20±0.08	Nil
10 %	Nil	Nil	2.70±0.04	3.50±0.10	Nil
20 %	1.80±0.04	3.10±0.07	4.40±0.11	5.30±0.16	Nil
Chlorhexidine (Std.)	2.40±0.16	3.20±0.22	4.60±0.32	5.90±0.41	-------

Values were expressed as Mean ± SD.

On the basis of above results, 20% concentrations of plant extract possess effective against bacteria as *Streptococcus mutans* and near to the standard.

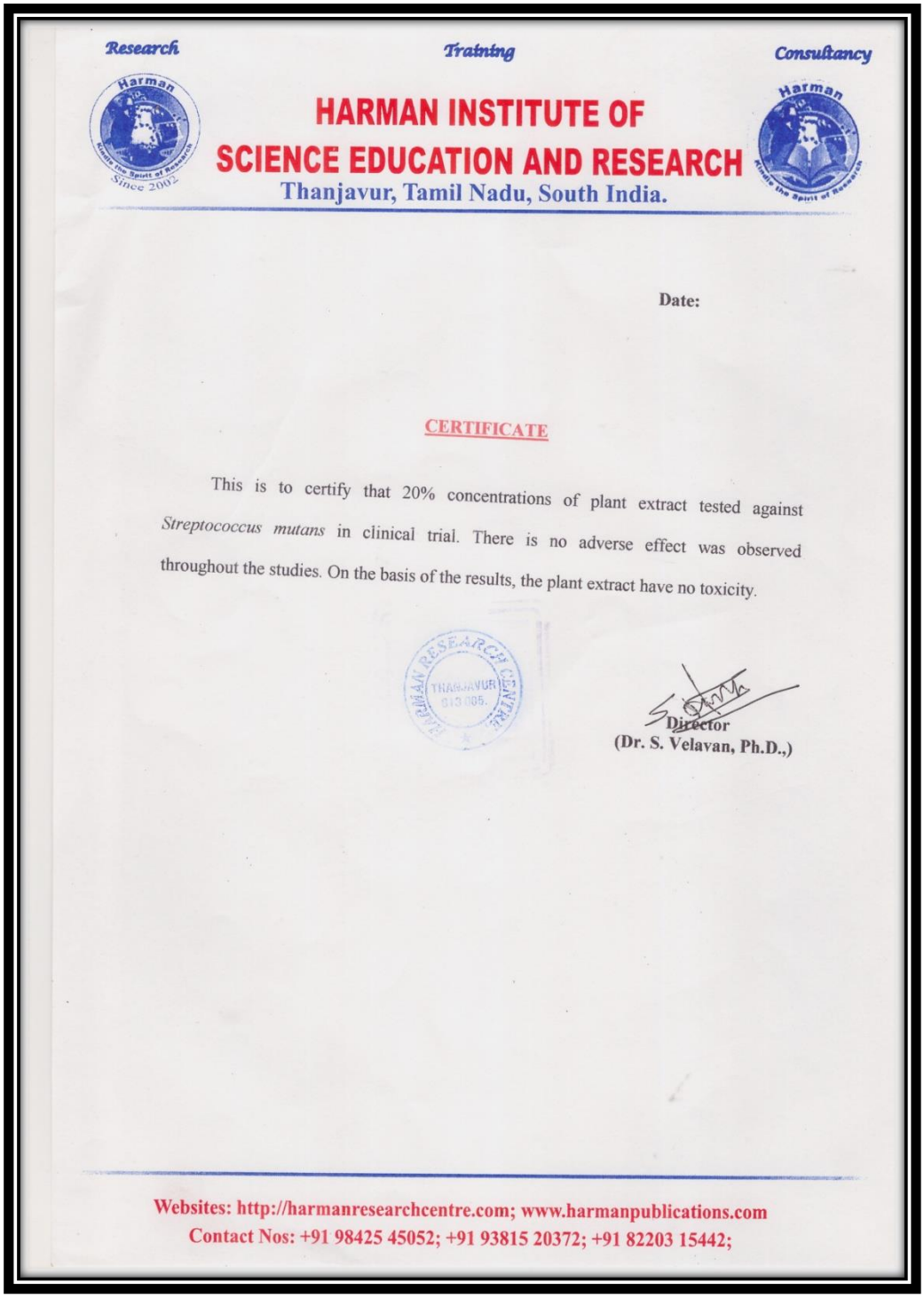
HARMAN RESEARCH CENTRE THANJAVUR 613 005.

Director
(Dr. S. Velavan, Ph.D.,)

Websites: http://harmanresearchcentre.com; www.harmanpublications.com
Contact Nos: +91 98425 45052; +91 93815 20372; +91 82203 15442;

ANNEXURE 6

CERTIFICATE FOR HERBAL MOUTHWASH TOXICITY



Research Training Consultancy

HARMAN INSTITUTE OF
SCIENCE EDUCATION AND RESEARCH
Thanjavur, Tamil Nadu, South India.

Date:

CERTIFICATE

This is to certify that 20% concentrations of plant extract tested against *Streptococcus mutans* in clinical trial. There is no adverse effect was observed throughout the studies. On the basis of the results, the plant extract have no toxicity.

Director
(Dr. S. Velavan, Ph.D.,)

Websites: http://harmanresearchcentre.com; www.harmanpublications.com
Contact Nos: +91 98425 45052; +91 93815 20372; +91 82203 15442;

ANNEXURE 7

CERTIFICATE FOR HERBAL MOUTHWASH SHELF LIFE

NATIONAL PHARMA
HOSPITAL & RESEARCH INSTITUTE

#1,Medical College – Vallam Road,
(Opp. south zone cultural centre)
Thanjavur – 613 004
Tamilnadu- India.

Tel: +91 4362 241241/ 241242
Mob: +91 95242 41241
E-mail: ino@nphri.com
Website: www.nphri.com

DATE: 13.07.2017

CERTIFICATE

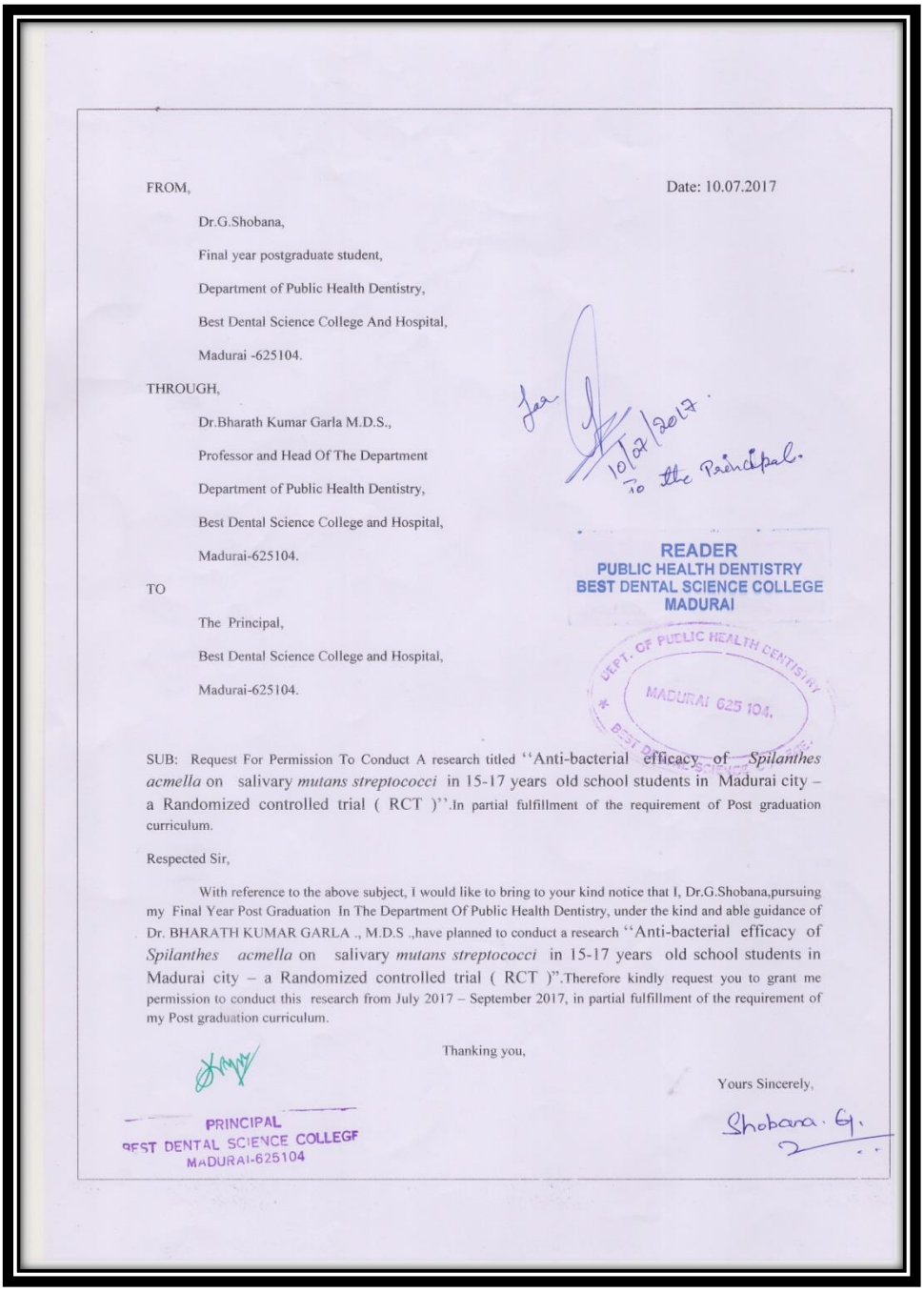
This is to certify that herbal mouthwash received from Dr.G.Shobana, studying final year post graduate student in Best dental science college, Madurai. The chemical analysis of phytochemicals & active ingredients of herbal mouthwash carried out in this research institute. 45 days shelf life of herbal mouthwash will be use for in vivo research and preserve it for 6 months.

For

NATIONAL PHARMA

ANNEXURE 8

LETTER OF ACCEPTANCE AND PERMISSION TO CONDUCT THE RESEARCH FROM PRINCIPAL AND HOD



FROM,

Dr.G.Shobana,

Final year postgraduate student,

Department of Public Health Dentistry,

Best Dental Science College And Hospital,

Madurai -625104.

Date: 10.07.2017

THROUGH,

Dr.Bharath Kumar Garla M.D.S.,

Professor and Head Of The Department

Department of Public Health Dentistry,

Best Dental Science College and Hospital,

Madurai-625104.

10/7/2017.
To the Principal.

READER
PUBLIC HEALTH DENTISTRY
BEST DENTAL SCIENCE COLLEGE
MADURAI

TO

The Principal,

Best Dental Science College and Hospital,

Madurai-625104.

DEPT. OF PUBLIC HEALTH DENTISTRY
MADURAI 625 104.

SUB: Request For Permission To Conduct A research titled ''Anti-bacterial efficacy of *Spilanthes acmella* on salivary *mutans streptococci* in 15-17 years old school students in Madurai city – a Randomized controlled trial (RCT)''.In partial fulfillment of the requirement of Post graduation curriculum.

Respected Sir,

With reference to the above subject, I would like to bring to your kind notice that I, Dr.G.Shobana,pursuing my Final Year Post Graduation In The Department Of Public Health Dentistry, under the kind and able guidance of Dr. BHARATH KUMAR GARLA ., M.D.S .,have planned to conduct a research ''Anti-bacterial efficacy of *Spilanthes acmella* on salivary *mutans streptococci* in 15-17 years old school students in Madurai city – a Randomized controlled trial (RCT)''.Therefore kindly request you to grant me permission to conduct this research from July 2017 – September 2017, in partial fulfillment of the requirement of my Post graduation curriculum.

Thanking you,

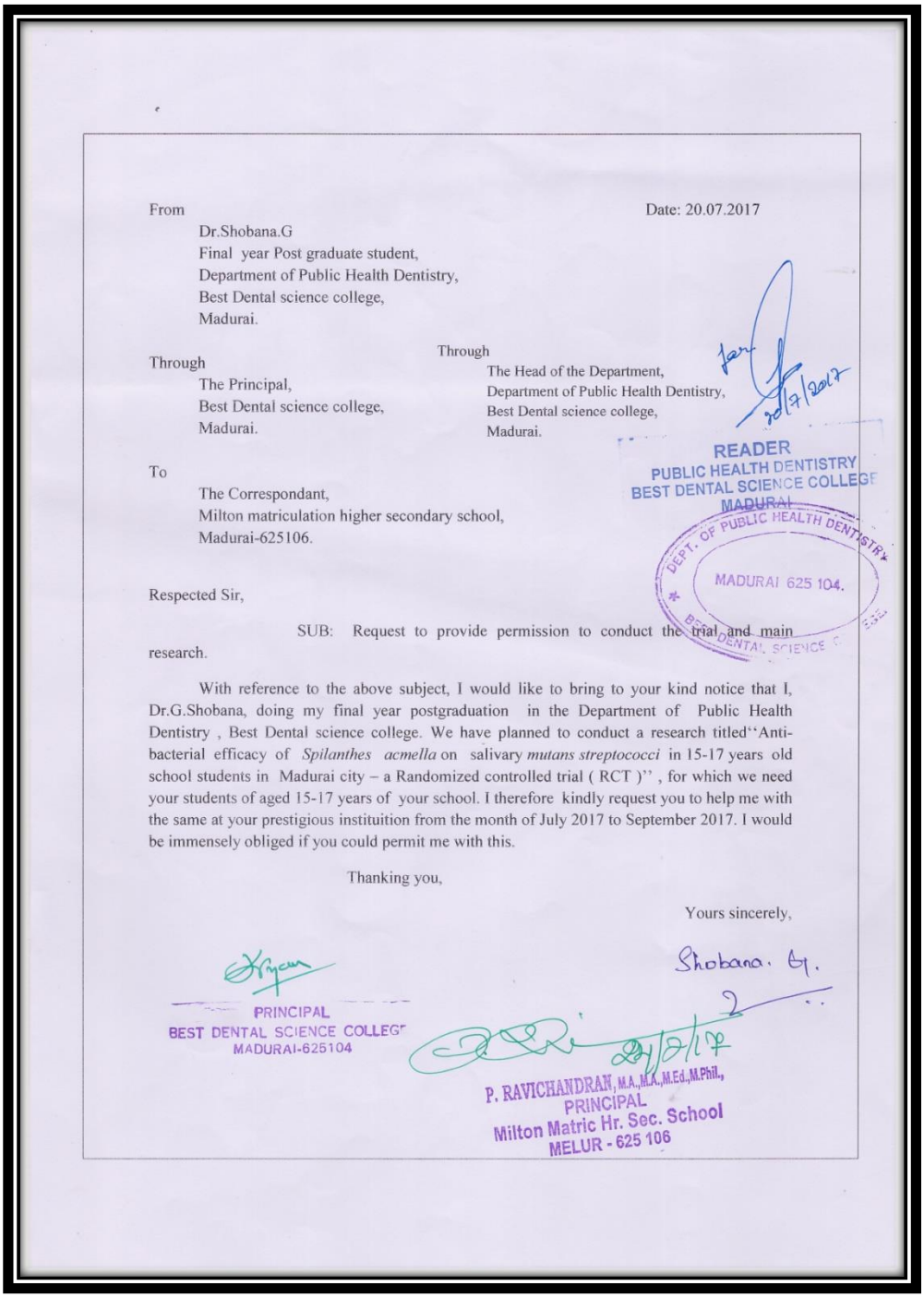
Yours Sincerely,

Shobana. G.

PRINCIPAL
BEST DENTAL SCIENCE COLLEGE
MADURAI-625104

ANNEXURE 9

LETTER OF ACCEPTANCE OF PILOT STUDY IN EDUCATIONAL INSTITUTION



From

Dr.Shobana.G
Final year Post graduate student,
Department of Public Health Dentistry,
Best Dental science college,
Madurai.

Date: 20.07.2017

Through
The Principal,
Best Dental science college,
Madurai.

Through
The Head of the Department,
Department of Public Health Dentistry,
Best Dental science college,
Madurai.

READER
PUBLIC HEALTH DENTISTRY
BEST DENTAL SCIENCE COLLEGE
MADURAI

DEPT. OF PUBLIC HEALTH DENTISTRY
MADURAI 625 104.
BEST DENTAL SCIENCE

To

The Correspondant,
Milton matriculation higher secondary school,
Madurai-625106.

Respected Sir,

SUB: Request to provide permission to conduct the trial and main research.

With reference to the above subject, I would like to bring to your kind notice that I, Dr.G.Shobana, doing my final year postgraduation in the Department of Public Health Dentistry , Best Dental science college. We have planned to conduct a research titled''Anti-bacterial efficacy of *Spilanthes acmella* on salivary *mutans streptococci* in 15-17 years old school students in Madurai city – a Randomized controlled trial (RCT)'' , for which we need your students of aged 15-17 years of your school. I therefore kindly request you to help me with the same at your prestigious instituition from the month of July 2017 to September 2017. I would be immensely obliged if you could permit me with this.

Thanking you,

Yours sincerely,

Shobana. G.

PRINCIPAL
BEST DENTAL SCIENCE COLLEGE
MADURAI-625104

P. RAVICHANDRAN, M.A.,M.A.,M.Ed.,M.Phil.,
PRINCIPAL
Milton Matric Hr. Sec. School
MELUR - 625 106

ANNEXURE 10

STUDY PROFORMA-PAGE-1

BEST DENTAL SCIENCE COLLEGE AND HOSPITAL

MADURAI

DEPARTMENT OF PUBLIC HEALTH DENTISTRY

STUDY TITLE: ''Anti-bacterial efficacy of *spilanthes acmella* on salivary *mutans streptococci* in 15-17 years old school students in Madurai city – a Randomized controlled trial (RCT)''

GUIDED BY	NAME OF THE INVESTIGATOR
Dr. R. Muthu Karuppaiah.,M.D.S.,	Dr. G. Shobana ,
Reader.	Final year PG student.

Case No.: Date:

Name:

Age/Gender:

Occupation:

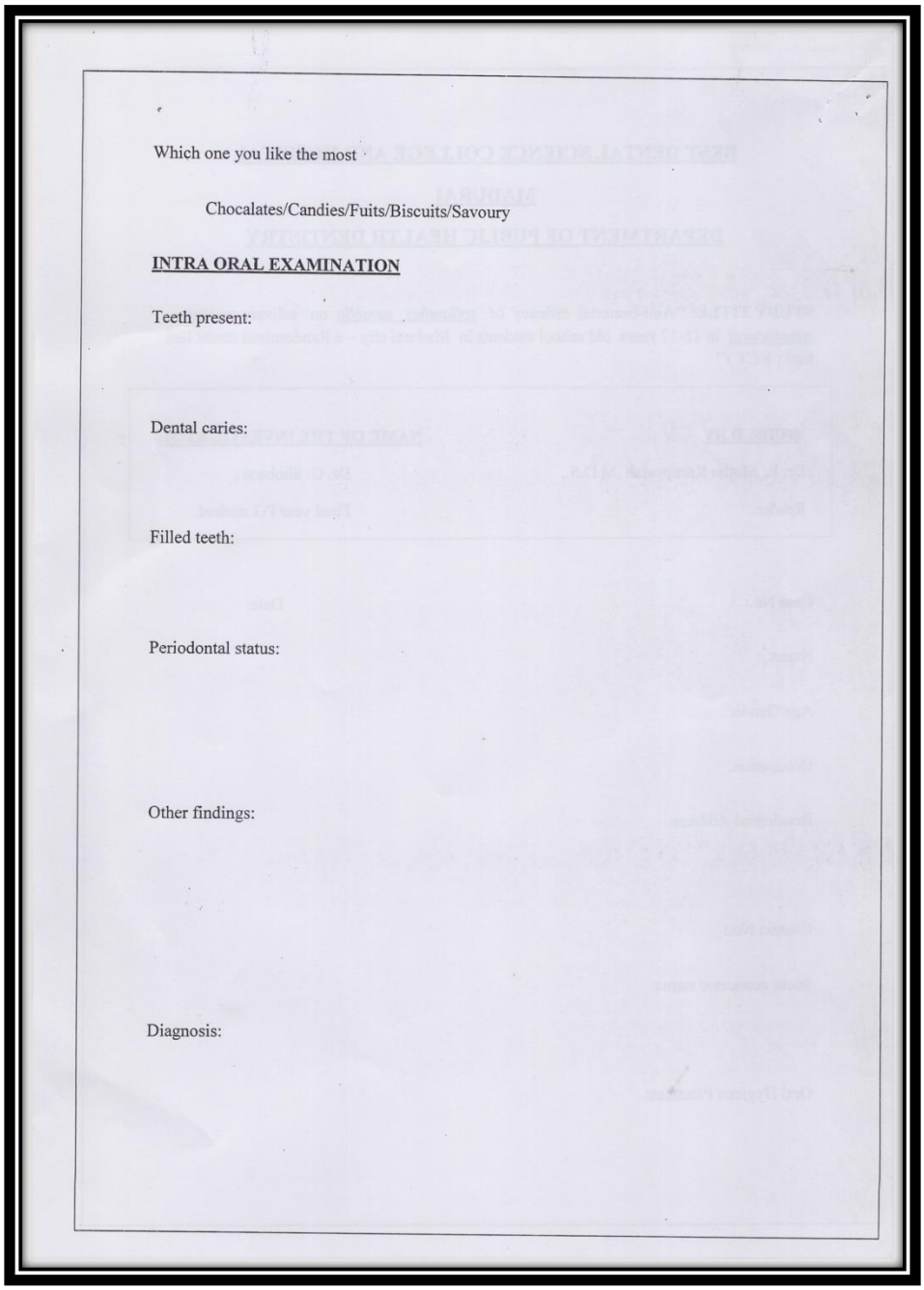
Residential Address:

Contact No.:

Socio economic status:

Oral Hygiene Practices:

STUDY PROFORMA-PAGE-2



Which one you like the most

Chocalates/Candies/Fuits/Biscuits/Savoury

INTRA ORAL EXAMINATION

Teeth present:

Dental caries:

Filled teeth:

Periodontal status:

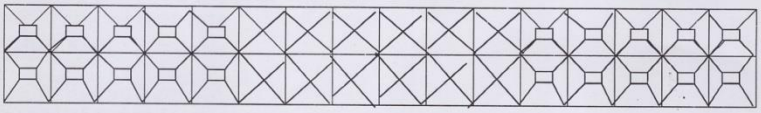
Other findings:

Diagnosis:

STUDY PROFORMA-PAGE-3

RECORD OF INDEX

Recording DMFS index

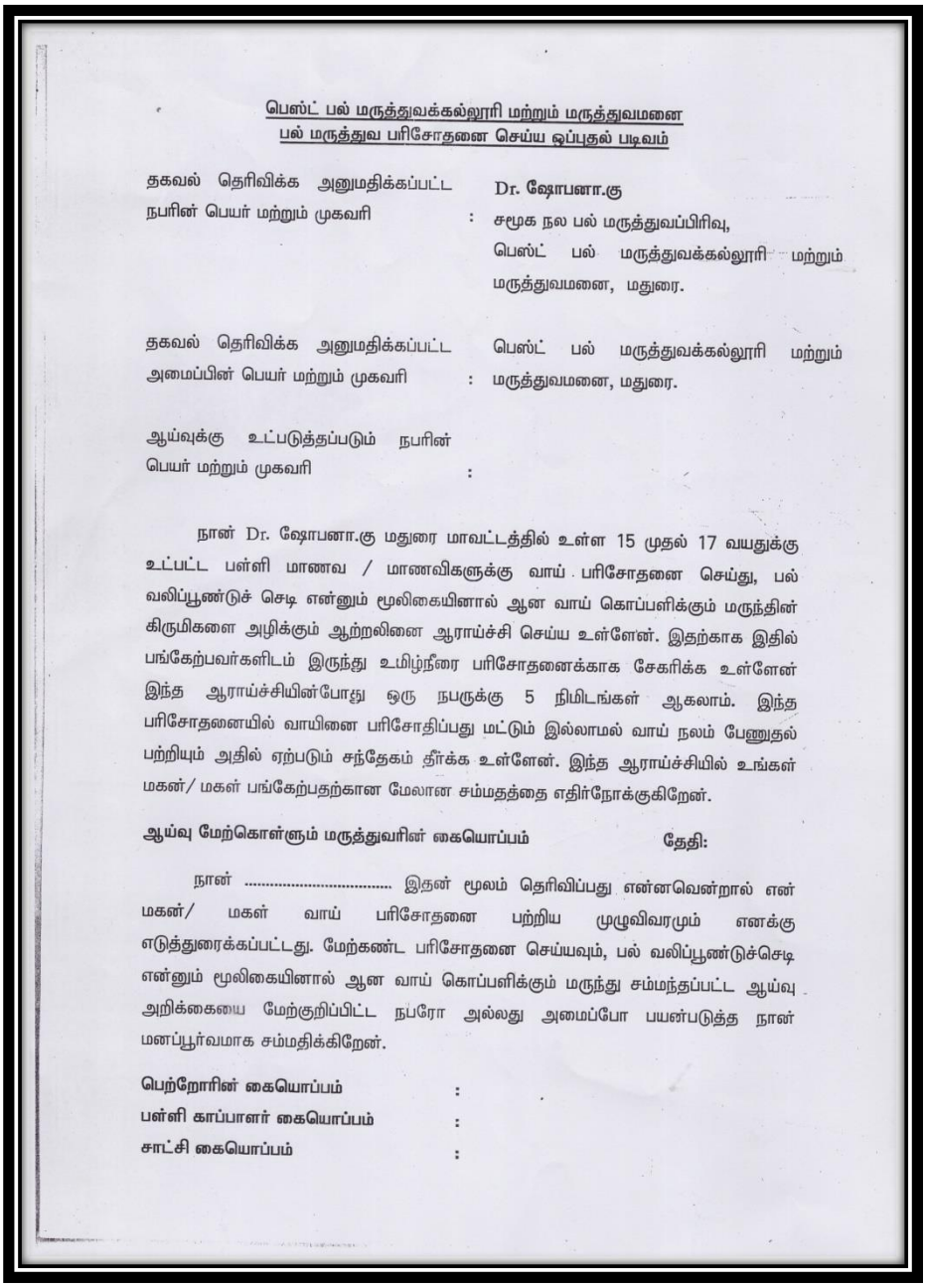


DS= MS= FS=

DMFS SCORE=

ANNEXURE 11

INFORMED CONSENT



பெஸ்ட் பல் மருத்துவக்கல்லூரி மற்றும் மருத்துவமனை
பல் மருத்துவ பரிசோதனை செய்ய ஒப்புதல் படிவம்

தகவல் தெரிவிக்க அனுமதிக்கப்பட்ட நபரின் பெயர் மற்றும் முகவரி : Dr. ஷோபனா.கு
சமூக நல பல் மருத்துவப்பிரிவு,
பெஸ்ட் பல் மருத்துவக்கல்லூரி மற்றும் மருத்துவமனை, மதுரை.

தகவல் தெரிவிக்க அனுமதிக்கப்பட்ட அமைப்பின் பெயர் மற்றும் முகவரி : பெஸ்ட் பல் மருத்துவக்கல்லூரி மற்றும் மருத்துவமனை, மதுரை.

ஆய்வுக்கு உட்படுத்தப்படும் நபரின் பெயர் மற்றும் முகவரி :

நான் Dr. ஷோபனா.கு மதுரை மாவட்டத்தில் உள்ள 15 முதல் 17 வயதுக்கு உட்பட்ட பள்ளி மாணவ / மாணவிகளுக்கு வாய் பரிசோதனை செய்து, பல் வலிப்பூண்டுச் செடி என்னும் மூலிகையினால் ஆன வாய் கொப்பளிக்கும் மருந்தின் கிருமிகளை அழிக்கும் ஆற்றலினை ஆராய்ச்சி செய்ய உள்ளேன். இதற்காக இதில் பங்கேற்பவர்களிடம் இருந்து உமிழ்நீரை பரிசோதனைக்காக சேகரிக்க உள்ளேன் இந்த ஆராய்ச்சியின்போது ஒரு நபருக்கு 5 நிமிடங்கள் ஆகலாம். இந்த பரிசோதனையில் வாயினை பரிசோதிப்பது மட்டும் இல்லாமல் வாய் நலம் பேணுதல் பற்றியும் அதில் ஏற்படும் சந்தேகம் தீர்க்க உள்ளேன். இந்த ஆராய்ச்சியில் உங்கள் மகன்/ மகள் பங்கேற்பதற்கான மேலான சம்மதத்தை எதிர்நோக்குகிறேன்.

ஆய்வு மேற்கொள்ளும் மருத்துவரின் கையொப்பம் **தேதி:**

நான் ..... இதன் மூலம் தெரிவிப்பது என்னவென்றால் என் மகன்/ மகள் வாய் பரிசோதனை பற்றிய முழுவிவரமும் எனக்கு எடுத்துரைக்கப்பட்டது. மேற்கண்ட பரிசோதனை செய்யவும், பல் வலிப்பூண்டுச்செடி என்னும் மூலிகையினால் ஆன வாய் கொப்பளிக்கும் மருந்து சம்மந்தப்பட்ட ஆய்வு அறிக்கையை மேற்குறிப்பிட்ட நபரோ அல்லது அமைப்போ பயன்படுத்த நான் மனப்பூர்வமாக சம்மதிக்கிறேன்.

பெற்றோரின் கையொப்பம் :
பள்ளி காப்பாளர் கையொப்பம் :
சாட்சி கையொப்பம் :

ANNEXURE 12

PILOT STUDY RESULTS

BEFORE INTERVENTION

Research *Training* *Testing* *Consultancy*

HARMAN INSTITUTE OF
SCIENCE EDUCATION AND RESEARCH
ISO 9001 : 2008 Certified Institution
Thanjavur
Websites: http://harmanresearchcentre.com; http://www.harmanpublications.com
Contact Nos: +919842545052; +919381520372; +918220315442;

Date: 04/08/2017

Results

Samples	Number of colonies	Results (CFU/ml)
A1	301	12.04×10^{6}
A2	236	9.44×10^{5}
A3	293	11.72×10^{5}
A4	262	10.48×10^{6}
A5	273	10.92×10^{6}
B1	98	3.92×10^{6}
B2	101	4.04×10^{5}
B 3	83	3.32×10^{5}
B 4	156	6.24×10^{6}
B 5	126	5.04×10^{6}
C 1	83	3.32×10^{6}
C 2	82	3.28×10^{5}
C 3	86	3.44×10^{5}
C 4	102	4.08×10^{6}
C 5	186	7.44×10^{6}
D 1	191	7.64×10^{6}
D 2	203	8.12×10^{5}
D 3	250	10.00×10^{5}
D 4	203	8.12×10^{6}
D 5	127	5.08×10^{6}

Director
(Dr. S. Velavan, Ph.D.)

PILOT STUDY RESULTS

AFTER INTERVENTION

Research Training Testing Consultancy

HARMAN INSTITUTE OF
SCIENCE EDUCATION AND RESEARCH
ISO 9001 : 2008 Certified Institution
Thanjavur

Websites: http://harmanresearchcentre.com; http://www.harmanpublications.com
Contact Nos: +919842545052; +919381520372; +918220315442;

Date:31/08/2017

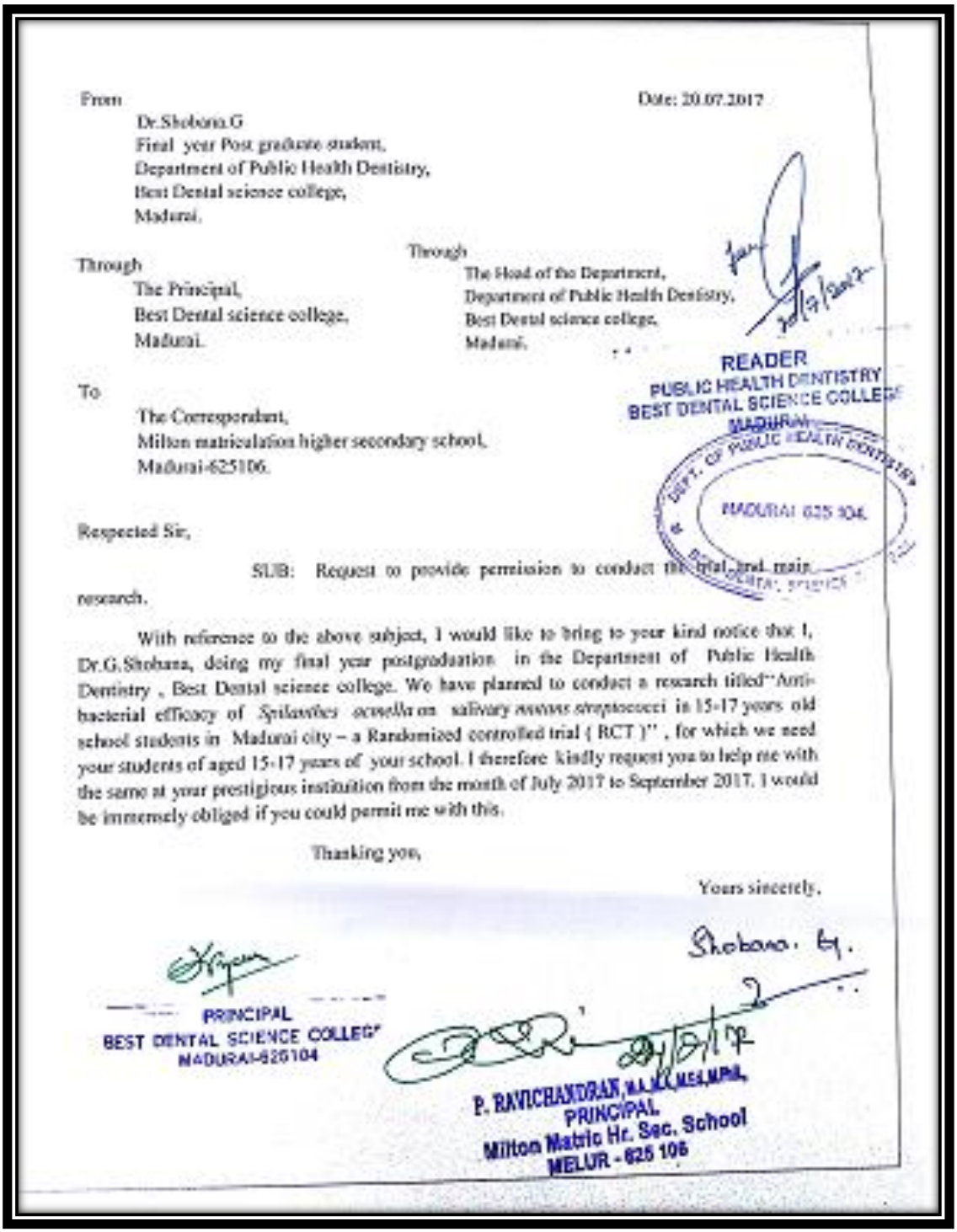
Results

Samples	Number of colonies	Results (CFU/ml)
A1	125	5.00×10^{6}
A2	73	2.92×10^{5}
A3	81	3.24×10^{5}
A4	119	4.76×10^{6}
A5	98	3.92×10^{6}
B1	67	2.68×10^{6}
B2	112	4.48×10^{5}
B 3	136	5.44×10^{5}
B 4	77	3.08×10^{6}
B 5	63	2.52×10^{6}
C 1	102	4.08×10^{6}
C 2	111	4.44×10^{5}
C 3	143	5.72×10^{5}
C 4	96	3.84×10^{6}
C 5	126	5.04×10^{6}
D 1	79	3.16×10^{6}
D 2	51	2.04×10^{5}
D 3	68	2.72×10^{5}
D 4	94	3.76×10^{6}
D 5	47	1.88×10^{6}

Director
(Dr. S. Velavan, Ph.D.,)

ANNEXURE 13

A. Milton Matriculation Higher Secondary School



From

Dr.Shobana.G
Final year Post graduate student,
Department of Public Health Dentistry,
Best Dental science college,
Madurai.

Date: 20.07.2017

Through

The Principal,
Best Dental science college,
Madurai.

Through

The Head of the Department,
Department of Public Health Dentistry,
Best Dental science college,
Madurai.

READER
PUBLIC HEALTH DENTISTRY
BEST DENTAL SCIENCE COLLEGE
MADURAI

DEPT. OF PUBLIC HEALTH DENTISTRY
MADURAI 625 104.
BEST DENTAL SCIENCE

To

The Correspondant,
Milton matriculation higher secondary school,
Madurai-625106.

Respected Sir,

SUB: Request to provide permission to conduct the trial and main research.

With reference to the above subject, I would like to bring to your kind notice that I, Dr.G.Shobana, doing my final year postgraduation in the Department of Public Health Dentistry , Best Dental science college. We have planned to conduct a research titled"Anti-bacterial efficacy of *Spilanthes acmella* on salivary *mutans streptococci* in 15-17 years old school students in Madurai city – a Randomized controlled trial (RCT)" , for which we need your students of aged 15-17 years of your school. I therefore kindly request you to help me with the same at your prestigious institution from the month of July 2017 to September 2017. I would be immensely obliged if you could permit me with this.

Thanking you,

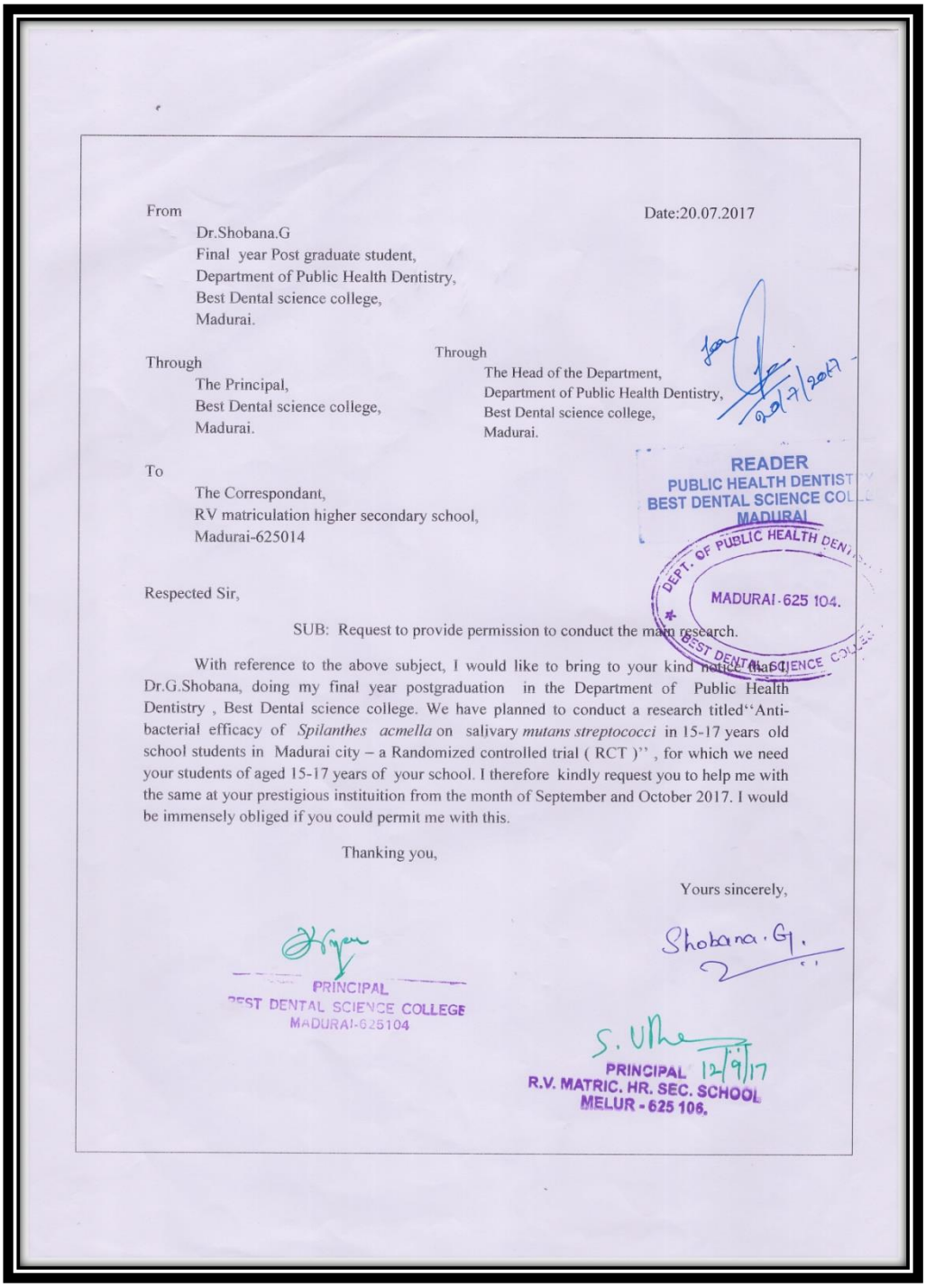
Yours sincerely,

Shobana. G.

PRINCIPAL
BEST DENTAL SCIENCE COLLEGE
MADURAI-625104

P. RAVICHANDRAN, M.A., M.Ed., M.Phil.,
PRINCIPAL
Milton Matric Hr. Sec. School
MELUR - 625 106

B. RV Matriculation Higher Secondary School,



From

Dr.Shobana.G
Final year Post graduate student,
Department of Public Health Dentistry,
Best Dental science college,
Madurai.

Date:20.07.2017

Through
The Principal,
Best Dental science college,
Madurai.

Through
The Head of the Department,
Department of Public Health Dentistry,
Best Dental science college,
Madurai.

READER
PUBLIC HEALTH DENTIST
BEST DENTAL SCIENCE COLLEGE
MADURAI

DEPT. OF PUBLIC HEALTH DENTISTRY
MADURAI-625 104.
BEST DENTAL SCIENCE COLLEGE

To

The Correspondant,
RV matriculation higher secondary school,
Madurai-625014

Respected Sir,

SUB: Request to provide permission to conduct the main research.

With reference to the above subject, I would like to bring to your kind notice that I, Dr.G.Shobana, doing my final year postgraduation in the Department of Public Health Dentistry , Best Dental science college. We have planned to conduct a research titled''Anti-bacterial efficacy of *Spilanthes acmella* on salivary *mutans streptococci* in 15-17 years old school students in Madurai city – a Randomized controlled trial (RCT)'' , for which we need your students of aged 15-17 years of your school. I therefore kindly request you to help me with the same at your prestigious instituition from the month of September and October 2017. I would be immensely obliged if you could permit me with this.

Thanking you,

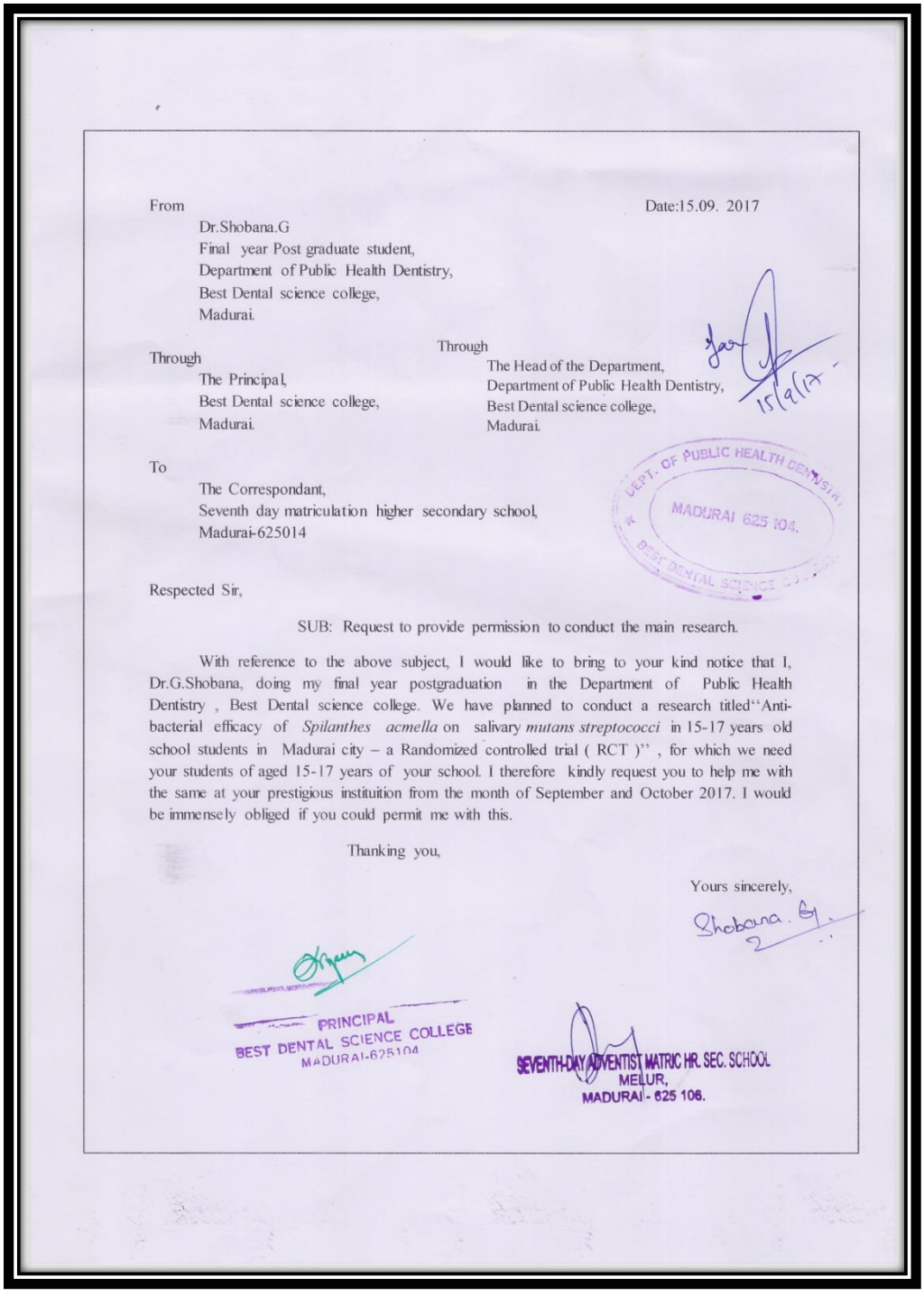
Yours sincerely,

Shobana. G.

PRINCIPAL
BEST DENTAL SCIENCE COLLEGE
MADURAI-625104

PRINCIPAL 12/9/17
R.V. MATRIC. HR. SEC. SCHOOL
MELUR - 625 106.

C. SEVENTHDAY MATRICULATION HIGHER SECONDARY SCHOOL,



From

Dr.Shobana.G
Final year Post graduate student,
Department of Public Health Dentistry,
Best Dental science college,
Madurai.

Date:15.09. 2017

Through
The Principal,
Best Dental science college,
Madurai.

Through
The Head of the Department,
Department of Public Health Dentistry,
Best Dental science college,
Madurai.

15/9/17

DEPT. OF PUBLIC HEALTH DENTISTRY
MADURAI 625 104.
BEST DENTAL SCIENCE COLLEGE

To

The Correspondant,
Seventh day matriculation higher secondary school,
Madurai-625014

Respected Sir,

SUB: Request to provide permission to conduct the main research.

With reference to the above subject, I would like to bring to your kind notice that I, Dr.G.Shobana, doing my final year postgraduation in the Department of Public Health Dentistry , Best Dental science college. We have planned to conduct a research titled''Anti-bacterial efficacy of *Spilanthes acmella* on salivary *mutans streptococci* in 15-17 years old school students in Madurai city – a Randomized controlled trial (RCT)'' , for which we need your students of aged 15-17 years of your school. I therefore kindly request you to help me with the same at your prestigious instituition from the month of September and October 2017. I would be immensely obliged if you could permit me with this.

Thanking you,

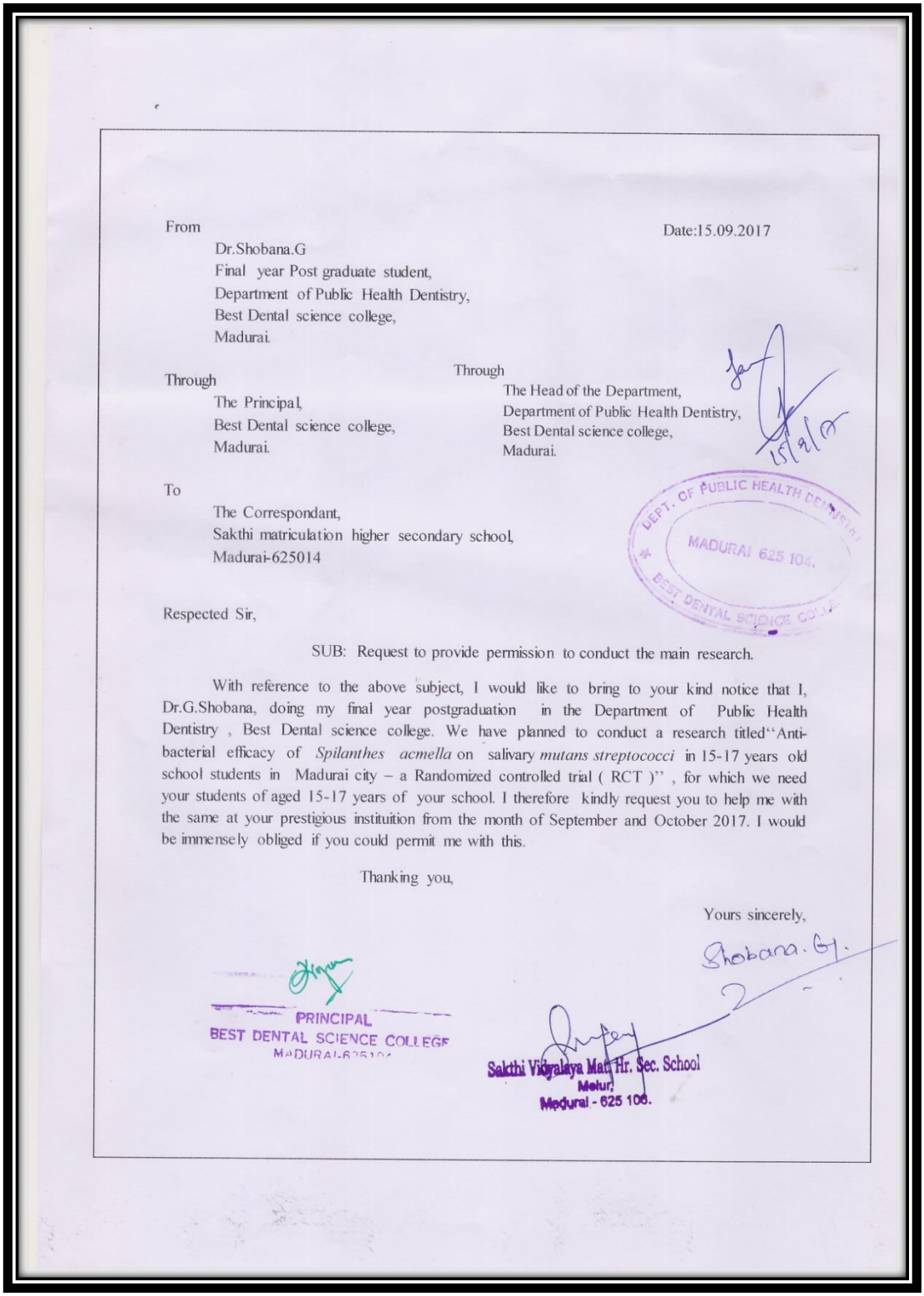
Yours sincerely,

Shobana. G

PRINCIPAL
BEST DENTAL SCIENCE COLLEGE
MADURAI-625104

SEVENTH-DAY ADVENTIST MATRIC HR. SEC. SCHOOL
MELUR,
MADURAI - 625 106.

D. SAKTHI MATRICULATION HIGHER SECONDARY SCHOOL



From

Dr.Shobana.G
Final year Post graduate student,
Department of Public Health Dentistry,
Best Dental science college,
Madurai.

Date:15.09.2017

Through
The Principal,
Best Dental science college,
Madurai.

Through
The Head of the Department,
Department of Public Health Dentistry,
Best Dental science college,
Madurai.

15/9/17

DEPT. OF PUBLIC HEALTH DENTISTRY
MADURAI 625 104.
BEST DENTAL SCIENCE COLLEGE

To

The Correspondant,
Sakthi matriculation higher secondary school,
Madurai-625014

Respected Sir,

SUB: Request to provide permission to conduct the main research.

With reference to the above subject, I would like to bring to your kind notice that I, Dr.G.Shobana, doing my final year postgraduation in the Department of Public Health Dentistry , Best Dental science college. We have planned to conduct a research titled"Anti-bacterial efficacy of *Spilanthes acmella* on salivary *mutans streptococci* in 15-17 years old school students in Madurai city – a Randomized controlled trial (RCT)" , for which we need your students of aged 15-17 years of your school. I therefore kindly request you to help me with the same at your prestigious instituition from the month of September and October 2017. I would be immensely obliged if you could permit me with this.

Thanking you,

Yours sincerely,

Shobana.G.

PRINCIPAL
BEST DENTAL SCIENCE COLLEGE
MADURAI-625104

Sakthi Vidyalaya Mat. Hr. Sec. School
Melur,
Madurai - 625 106.

ANNEXURE 14

FEEDBACK FORM

FEEDBACK FORM | **SERIAL NO.**

School name:

Student name: **Age/gender:**

Standard:

Study title: Anti-bacterial efficacy of Spilanthes acmella on salivary mutans Streptococci in 15-17 years old students in Madurai city – A Randomized Controlled Trial (RCT).

1. Did you use mouthwash regularly?

   a) Yes b) No

   If Yes, Reason……

2. How did you feel while using mouthwash?

   a) Freshens b) Worsens c) No change

3. Did you feel any of the following discomfort while using mouthwash?

   a) Burning sensation in the oral cavity b) Oral ulcer c) Taste alteration

   d) Any other……

4. How was the taste of mouthwash?

   a) Pleasant b) Unpleasant

5. Did you feel any of the following discomfort in general health while using mouthwash

   a) Fever b) Vomiting c) Diarrhoea

   d) Any other……

ANNEXURE 15

RESULTS OF MAIN STUDY-GROUP A

PAGE-1

Research Training Testing Consultancy

HARMAN INSTITUTE OF
SCIENCE EDUCATION AND RESEARCH
ISO 9001 : 2008 Certified Institution
Thanjavur

Websites: http://harmanresearchcentre.com; http://www.harmanpublications.com
Contact Nos: +919842545052; +919381520372; +918220315442;

Date: 10/11/2017

Results

Samples	Results (CFU/ml) BEFORE	Results (CFU/ml) AFTER
A1	12.16×10^6	3.2×10^6
A2	10.92×10^6	2.86×10^6
A3	10.94×10^6	2.92×10^6
44	9.6×10^6	3.64×10^6
A5	9.88×10^6	3.08×10^6
A6	6.16×10^6	3.28×10^6
A7	12.04×10^6	3.2×10^6
A8	5×10^6	3.88×10^6
A9	7.56×10^6	3.48×10^6
A10	8.4×10^6	3.88×10^6
A11	9.44×10^6	2.92×10^6
A12	7.64×10^6	2.16×10^6
A13	8.12×10^6	2.04×10^6
A14	5.08×10^6	3.84×10^6
A15	11.72×10^6	2.76×10^6
A16	10×10^6	2.88×10^6
A17	11.24×10^6	2.16×10^6
A18	10.48×10^6	2.78×10^6
A19	8.12×10^6	2.08×10^6
A20	12.04×10^6	2.76×10^6

Director
(Dr. S. Velavan, Ph.D.,)

RESULTS OF MAIN STUDY-GROUP B

PAGE-2

Research Training Testing Consultancy

HARMAN INSTITUTE OF
SCIENCE EDUCATION AND RESEARCH
ISO 9001 : 2008 Certified Institution
Thanjavur

Websites: http://harmanresearchcentre.com; http://www.harmanpublications.com
Contact Nos: +919842545052; +919381520372; +918220315442;

Date: 10/11/2017

Results

Samples	Results (CFU/ml) BEFORE	Results (CFU/ml) AFTER
B1	10.92×10^6	4.66×10^6
B2	6.64×10^6	3.72×10^6
B3	7.01×10^6	3.88×10^6
B4	6.08×10^6	3.18×10^6
B5	7.04×10^6	3.22×10^6
B6	5.92×10^6	2.68×10^6
B7	8.24×10^6	4.8×10^6
B8	7.2×10^6	3.36×10^6
B9	9.44×10^6	4.42×10^6
B10	8.56×10^6	4.34×10^6
B11	9.16×10^6	3.84×10^6
B12	11.72×10^6	5.86×10^6
B13	7.76×10^6	3.22×10^6
B14	9.88×10^6	4.32×10^6
B15	9.8×10^6	4.38×10^6
B16	8.68×10^6	3.22×10^6
B17	9.96×10^6	5.12×10^6
B18	9.08×10^6	4.88×10^6
B19	7.96×10^6	4.98×10^6
B20	6.32×10^6	3.88×10^6

Director
(Dr. S. Velavan, Ph.D.,)

RESULTS OF MAIN STUDY-GROUP C

PAGE-3

Research Training Testing Consultancy

HARMAN INSTITUTE OF
SCIENCE EDUCATION AND RESEARCH
ISO 9001 : 2008 Certified Institution
Thanjavur

Websites: http://harmanresearchcentre.com; http://www.harmanpublications.com
Contact Nos: +919842545052; +919381520372; +918220315442;

Date: 10/11/2017

Results

Samples	Results (CFU/ml) BEFORE	Results (CFU/ml) AFTER
C1	7.96×10^6	5.8×10^6
C2	7.32×10^6	5.08×10^6
C3	8.96×10^6	6.64×10^6
C4	9.44×10^6	7.28×10^6
C5	9.08×10^6	8.02×10^6
C6	8.92×10^6	6.98×10^6
C7	9.2×10^6	7.88×10^6
C8	8.8×10^6	6.96×10^6
C9	8.44×10^6	6.84×10^6
C10	8.56×10^6	7.22×10^6
C11	8.36×10^6	7.66×10^6
C12	8.68×10^6	6.4×10^6
C13	9.85×10^6	7.28×10^6
C14	8.16×10^6	7.84×10^6
C15	8.24×10^6	7.12×10^6
C16	8.28×10^6	6.86×10^6
C17	8.44×10^6	7.4×10^6
C18	8.42×10^6	6.98×10^6
C19	8.8×10^6	6.96×10^6
C20	8.68×10^6	6.28×10^6

Director
(Dr. S. Velavan, Ph.D.,)

RESULTS OF MAIN STUDY-GROUP D

PAGE-4

Research Training Testing Consultancy

HARMAN INSTITUTE OF
SCIENCE EDUCATION AND RESEARCH
ISO 9001 : 2008 Certified Institution
Thanjavur

Websites: http://harmanresearchcentre.com; http://www.harmanpublications.com
Contact Nos: +919842545052; +919381520372; +918220315442;

Date: 10/11/2017

Results

Samples	Results (CFU/ml) BEFORE	Results (CFU/ml) AFTER
D1	8.68×10^6	2.28×10^6
D2	8.24×10^6	2.88×10^6
D3	8.48×10^6	3.76×10^6
D4	9.58×10^6	2.2×10^6
D5	9.6×10^6	3.8×10^6
D6	9.88×10^6	3.72×10^6
D7	5.08×10^6	1.88×10^6
D8	6.96×10^6	2.04×10^6
D9	9.76×10^6	2.48×10^6
D10	10×10^6	2.72×10^6
D11	10.16×10^6	3.68×10^6
D12	9.96×10^6	3.32×10^6
D13	9.12×10^6	2.04×10^6
D14	10.04×10^6	2.68×10^6
D15	9.36×10^6	2.76×10^6
D16	8.92×10^6	3.3×10^6
D17	9.64×10^6	2.16×10^6
D18	9.12×10^6	3.76×10^6
D19	8.96×10^6	2.12×10^6
D20	10.72×10^6	3.08×10^6

Director
(Dr. S. Velavan, Ph.D.,)

ANNEXURE 16

LETTER OF ACCEPTANCE OF THE STATISTICIAN TO HELP WITH THE STATISTICAL ANALYSIS OF THERESEARCH

From

Mr.Asaithambi,M.Sc.,D.P.D,D.J,M.C

(Retd)Lecturer In Statistics And Demography

Research Officer,

ICMR, Madurai Medical College,

Madurai

To

The Head Of The Department

Department Of Public Health Dentistry

Best Dental Science College

Madurai

Respected Sir,

Sub: Acceptance To Help Your Student With Statistical Analysis Reg.

With reference to the above Subject, I Hereby Accept To Help Your Student, Dr.G. Shobana with the Statistical Analysis Of Her Work Titled **"Anti-Bacterial Efficacy Of Spilanthes Acmella On Salivary Mutans Streptococci In 15-17 Years Old School Students In Madurai District- A Randomized Controlled Trial (RCT) ".**

Thanking You,

Yours,

PROFESSOR & H.O.D.,
DEPT.OF PUBLIC HEALTH DENTISTRY,
BEST DENTAL SCIENCE COLLEGE,
MADURAI-625104.